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SCIENTIFIC CRITERIA DOCUMENT FOR DEVELOPMENT OF PROVINCIAL WATER QUALITY OBJECTIVES AND GUIDELINES

RESIN ACIDS

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Environment Ontario

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Errata Sheet

The following changes refer to information in the Resin Acids Criteria Development Document (ISBN 0-7729-4347-8):

Page 12, last line should read 12 000 kilograms (kg) not tons.

Page 30, footnote c (sockeye salmon) refers to the 0.65 ug/ml water concentration exposure data of Kruzynski (1979) at the bottom of Table 3.

These changes do not alter the numerical limits recommended for Provincial Water Quality Guidelines.

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This document is primarily the work of Lewis Yeager and Barry Taylor, formerly of EAG Ltd. They were assisted with review and scientific advice by Dr. George Dixon of Waterloo University. (His general comments are included as Appendix 3)

The draft was then distributed to approximately 60 scientific experts and interested groups for peer review. Responses were received and greatly appreciated from H. Samant and K. Doe, Environment Canada, Atlantic Region; G. Ozborne, Lakehead University; R. Voss and T. Kovaks, Pulp and Paper Institute; J. Betts and T. Ruthman, Environment Canada, Ottawa; R. Watts, Environment Canada, Pacific Region; J. Servizi, Fisheries and Oceans Canada; J.D. Kinkead, G. Myslik, and J.A. Murphy Ontario Ministry of the Environment.

The document was then revised based on the peer review by Scott Abernethy and Gary Westlake of the Aquatic Toxicity Unit. This final version includes approved Provincial Water Quality Guidelines for resin acids recommended by Water Management Policy Working Group I.

SUMMARY

Resin acids are extracted from the wood of coniferous trees, either naturally or as a result of activities of forest products industries, the quantities of each of the resin acids varying considerably with species of tree. The separation and chemical analysis of resin acids, which, a few years ago were difficult, particularly in samples from pulp and paper mill effluents, have improved. It is now also feasible to measure resin acid concentrations in fish tissues. Resin acids are readily biodegraded by a number of fungal and bacterial species and are normally broken down both in receiving waters and in pulp mill secondary treatment facilities by a group of organisms.

Dehydroabietic Acid (DHA) is not the most toxic but is the most persistent resin acid and is normally the one most common in the water column near pulp mills. Resin acids are hydrophobic, non-volatile compounds with a relatively high affinity for solids which, over time, results in substantial contamination of lake and river sediments. Their persistence in the bottom sediment of receiving environments is much greater than in the water column (a 21 year half-life has been estimated). This has important implications for sediment and pulp and paper mill sludge management. Acute lethal concentrations for salmonid fish are the lowest of the literature values that were found. Acute lethality of the individual resin acids seems to fall within a narrow range of 0.2 to 1.7 mg/L at neutral pH. Toxicity is highly dependent on pH. DHA, for example, is 15 to 30 times more toxic at pH 6.5 than at 9 making it necessary to set Provincial Water Quality Guidelines which reflect the receiving water pH. The resin acid Guidelines are as follows:

Receiving Water pH	Concentration (ug/L) DHA	Total Resin Acids
5.0[a]	1	1
5.5[a]	2	3
6.0[a]	2	4
6.5	4	9
7.0	8	25
7.5	12	45
8.0	13	52
8.5	14	60
9.0	14	62

a - lower than established PWQO for pH

SOMMAIRE

Les divers acides résineux sont extraits du bois des conifères, soit de façon naturelle, soit à la suite d'activités entreprises par les industries forestières. Leur quantité varie considérablement d'une espèce d'arbre à une autre. Il y a quelques années, séparer et analyser les acides résineux était particulièrement difficile, surtout dans le cas des échantillons provenant des effluents des usines de pâtes et papiers, mais les choses se sont améliorées depuis. Aujourd'hui, on peut même mesurer leurs concentrations dans la chair des poissons. Ces acides sont biodégradables; ils peuvent être mangés par des champignons ou des bactéries ou détruits par divers organismes dans les eaux réceptrices et les installations de traitement secondaire des usines de pâtes et papiers.

L'acide déhydroabiétique n'est pas l'acide le plus toxique, mais il est le plus persistant. C'est lui que l'on retrouve le plus souvent dans les colonnes d'eau près des usines de pâtes et papiers. Les acides résineux sont des composés hydrophobes et non volatils qui se fixent généralement sur les matières solides, ce qui, à long terme, se traduit par un taux élevé de pollution des sédiments dans les lacs et rivières. Ils demeurent beaucoup plus longtemps dans les sédiments des milieux récepteurs que dans la colonne d'eau (on estime qu'ils possèdent l'équivalent de 21 demi-vies). C'est un facteur très important dans la gestion des sédiments et des boues produites par les usines de pâtes et papiers. Les concentrations les moins élevées, d'après les recherches, sont celles mesurées chez les salmonidés. Il semblerait que l'effet létal aigu de chaque acide résineux n'oscille qu'entre 0,2 et 1,7 mg/L, à un pH neutre; la toxicité dépend énormément du pH. Ainsi, l'acide déhydroabiétique est de 15 à 30 fois plus toxique à un pH de 6,5 qu'il ne l'est à un pH de 9. Il est donc essentiel d'élaborer des lignes directrices provinciales sur la qualité de l'eau qui tiennent compte du pH des eaux réceptrices. Voici d'ailleurs ces lignes directrices :

	Concentration (µg/L)	
pH des eaux réceptrices	Acide déhydroabiétique	Acides résineux
5,0[a]	1	1
5,5[a]	2	3
6,0[a]	2	4
6,5	4	9
7,0	8	25
7,5	12	45
8,0	13	52
8,5	14	60
9,0	14	62

a - inférieur à la ligne directrice provinciale relative au pH

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1. INTRODUCTION

1.1 ONTARIO'S SURFACE WATER QUALITY OBJECTIVES AND GUIDELINES

Provincial Water Quality Objectives and Guidelines are a set of numerical criteria developed for individual substances or classes of substances primarily for the protection of aquatic life. Where warranted, an Objective is set to protect wildlife and humans consuming contaminated aquatic species. Similarly, if information indicates that taste or odour of water or tainting of fish flesh may be of concern, an Objective is set to ensure these factors do not affect water use. Objectives and Guidelines are established in accordance with policies described in the Ministry's Water Management Policy Manual (MOE, 1984), and are used to interpret data on the concentration of contaminants found in receiving waters and, indirectly, to assist with the control of discharges.

An extensive review and critical evaluation of all available scientific literature on a substance is required to determine its persistence, bioaccumulation potential, toxicity to aquatic organisms and organoleptic effects. This report is intended to synthesize the presently available data on toxicity and fates in aquatic environments of resin acids in Ontario, to provide a basis upon which Provincial Water Quality Objectives or Guidelines may be established.

1.2 BACKGROUND

Resin acids are diterpenoid carboxylic acids derived from the natural resins found in wood of coniferous trees. They are released into receiving waters as "extractives" from any processing of softwood trees, but especially from the pulp and paper industry. Resin acids are significant and primary sources of toxicity to fish in Canadian softwood pulping effluents (McLeay 1986), and have been linked to as much as 70% of the toxicity of whole effluents (Leach and Thakore 1977).

The significance of resin acids to the toxicity of pulp mill effluents has been known or suspected for a long time (Walden and Howard 1981, Rogers et al. 1975), but it is only since the early 1970's that the analytical procedures (such as the use of macroreticular resins) to identify and quantify individual resin acids in effluents have become available (Chung et al. 1979). Thus, although the toxicity of whole pulp mill effluents has been thoroughly studied (see reviews of Davis 1976, Poole et al. 1978, Kovacs 1986), the literature pertaining separately to resin acid toxicity is small.

A recent and thorough review of aquatic toxicity of pulp and paper mill effluents has been completed by McLeay & Assoc. (1986). That report documents the various mill processes, the nature of their effluents, and the toxicity of those effluents to freshwater and marine life, including chronic and sublethal effects, as well as bioaccumulation.

Toxicity of resin acids was considered by McLeay & Assoc. (1986) only in the context of their contribution to toxicity of whole mill effluents. The aim of the present report is to refine and extend the analysis in the foregoing report with respect to resin acids alone, with the ultimate end of establishing no-effect levels in surface water. Areas covered in detail by McLeay & Assoc. (1986) will be treated only briefly here, and the reader is referred to the former report for a fuller discussion.

Specific objectives of this report are:

- to review sources, transport, partitioning, persistence and degradation of resin acids (and their breakdown products) in surface fresh water, sediments and aquatic biota;
- to review and evaluate present knowledge of resin acid toxicity to all forms of freshwater life, including acute, chronic, lethal and sublethal effects, genetic effects, bioaccumulation, and flesh tainting;
- to evaluate the influence of water quality on the toxicity of resin acids;
- to compare toxicities, where known, among individual resin acids, and to predict toxicities of untested acids based on those comparisons;
- to propose water quality objectives which, based on present knowledge, are sufficient to protect aquatic ecosystems exposed to resin acids.

1.3 CHEMICAL ANALYSIS OF RESIN ACIDS

There are six resin acids commonly found in softwood pulp mill effluents: abietic, dehydroabietic, neoabietic, pimaric, isopimaric, and sandaracopimaric acids. Two others, levopimaric (Cherwinsky and Murray 1986, Servizi and Gordon 1986) and palustric (NCASI 1985, Leach and Thakore 1976, Holmbom and Lethinen 1980) are often included. All eight acids are found in pulp mill effluents in Canada (McLeay & Assoc. 1986). In addition there may be other, rarer compounds such as 7-oxodehydroabietic acid (Brownlee and Strachan 1977), 7,15-isopimaric, 8,15-isopimaric acids (Walden and Howard 1981), abietatetraenoic, and hydroxydehydroabietic acids (Rogers et al. 1979), which are present only in trace quantities.

The ensuing discussion is confined to the eight resin acids which may be present in quantities of greatest toxicological significance, and related compounds such as sodium salts of the acids (Couillard 1981) or degradation products. Figure 1 shows chemical structures for the eight resin acids considered here.

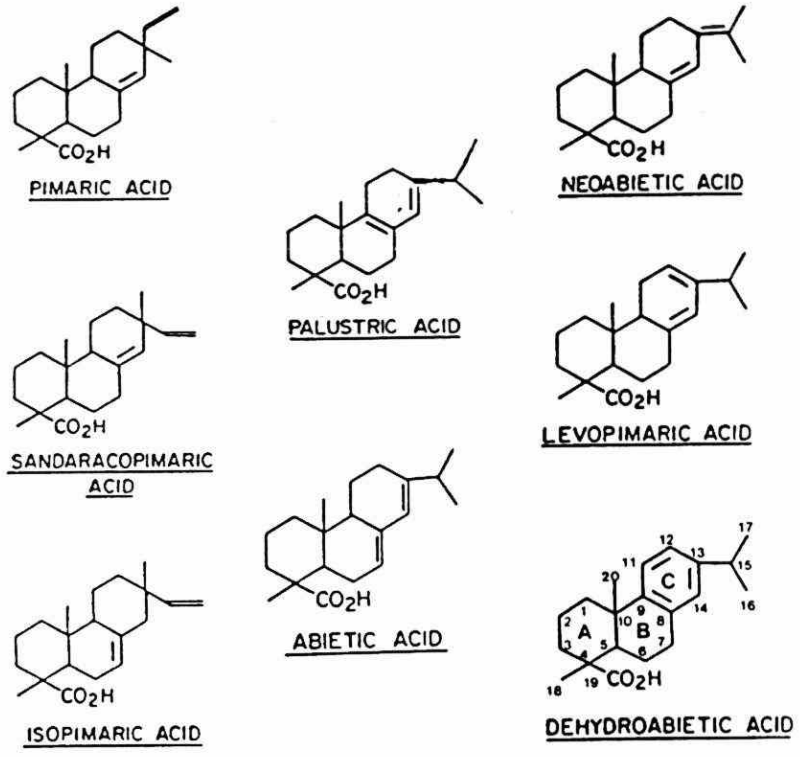

Figure 1. Structure of Resin Acids Found in Pulp and Paper Mill Effluents.

Of the resin acids, dehydroabietic acid has been shown to form chlorinated derivatives by electrophilic substitution of chlorine (used in pulp bleaching) into the aromatic ring. Chlorinated resin acids (two monochloro- and one dichloro-derivatives) are decidedly more persistent and more toxic than their parent compounds (McLeay & Assoc. 1986). In fact, they are sufficiently more hydrophobic to be treated as a distinct group for regulatory purposes, and this discussion is restricted to the nonchlorinated forms.

Separation of resin acids in effluent samples may involve any of a number of chromatographic procedures, including column chromatography (Leach and Thakore 1973), thin-layer chromatography (McKague et al. 1977), liquid chromatography (Kinae et al. 1981a), and gas-liquid chromatography (Leach and Thakore 1975, Brownlee et al. 1977). Once the acids have been separated, identification may use spectrometry, including infrared, ultraviolet, nuclear magnetic resonance and mass spectrometry, (Leach and Thakore 1973, Walden and Howard 1981). Computer-controlled gas chromatography, coupled with mass

spectrometry (GC-MS) is now the most widely used technique (Keith 1976, Rogers et al. 1979, Oikari et al. 1980).

The first step in analysis of pulp mill effluent is separation of the weak acids (resin and fatty acids) from the raw effluent. There are two approaches commonly used for this step. In the first, developed by the National Council of the Paper Industry for Air and Stream Improvement (NCASI), the effluent is acidified to pH 2-3, and acids are extracted in diethyl ether solvent. In the other methods, used by B.C. Research (Leach and Thakore 1973, 1975, 1977), the acids are extracted under alkaline conditions (pH 9-10) by adsorption onto a porous polymeric resin called Amberlite XAD-2. Dissolved material adsorbed to the resin is then eluted first with diethyl ether and then methanol. In some analyses, resin and fatty acids may need further purification by extraction with NaOH, to remove neutral contaminants (Leach and Thakore 1973, 1977). The extracted samples can then be subjected to GC-MS, after reaction with diazomethane to produce methyl ester derivatives (eg. Voss and Rapsomatiotis 1985), to separate individual compounds. Routine quantitative analysis for resin acids in pulp and paper mill effluents is however most often carried out by GC using a flame ionization detector (FID).

Analysis of resin acids in pulp mill effluents is not without problems. The solvent extraction method may produce foaming emulsions, which requires centrifugation to break. This increases sample preparation time, and may result in poor precision and low recovery. Rendering the effluent very acidic prevents the formation of emulsions, but often leads to isomerization of some resin acids, particularly levopimaric, (Mahood and Rogers 1975) and co-precipitation of lignin-acid complexes. Both problems, emulsions and precipitates, are more severe in samples in which the lignin content is high (Voss and Rapsomatiotis 1985).

The resin adsorption procedure is designed to overcome all of the limitations of solvent extractions, since there is no solvent with which to form emulsions, and pH is kept very alkaline. Notwithstanding, comparisons of resin and solvent methods sometimes show lower recovery by the resin method; furthermore, the recovery efficiencies of both methods and the pH of optimal recovery vary widely according to the kind of mill effluent sampled (i.e. bleached or unbleached, chemical or mechanical). Voss and Rapsomatiotis (1985) have recently proposed a new solvent extraction procedure using large volumes of methyl tert-butyl ether at pH 9, which may circumvent the problems of low- pH extractions. However, their method is too new to have been widely applied.

There have, in the past, also been problems in the GC phase of analysis. Many fatty acids elute simultaneously with resin acids, obscuring the peak area of the latter (J. Betts, Environment Canada, personal communication). The resin acids themselves have also been difficult to separate. Pimaric and sandaracopimaric acids produce close peaks under some circumstances as do abietic and dehydroabietic. Isopimaric, palustric, and levopimaric acids may also elute close together, and complete separation of levopimaric from palustric has often been difficult to achieve (Voss and Rapsomatiotis 1985).

Fortunately, modern advances in chromatography, especially the development of high resolution capillary GC columns, have permitted much finer resolution of organic mixtures, and consequently separation of individual resin acids and resin acids from fatty acids is now possible. However, many published data, especially in the "older" literature, include only values for resin acid pairs, e.g. palustric + levopimaric, or pimaric + sandaracopimaric (Maenpaa et al. 1968, Rogers and Mahood 1974, McKague et al. 1977).

It must be emphasized that these past difficulties of extraction and separation apply only to the complex chemical environment of pulp mill effluents. Assaying solutions of one or a few resin acids, as was necessary in the laboratory toxicity tests described in this report, is a relatively straightforward matter, and accuracy sufficient to ensure reliable toxicity estimates is easily attained.

A procedure for resin acid analysis of fish tissues has been developed by Oikari and co-workers in Finland (Oikari et al. 1980, 1984b). Tissue samples are excised from freshly killed fish and freeze-dried. Dry samples are then ground with sodium sulphate, acidified with sulphuric acid (pH 3-4), and refluxed for 5 hours with n-hexane. Alternatively, thawed tissues may be homegenized in water before being acidified and extracted with hexane (Oikari et al. 1982). Fox et al. (1977) used hexane-diethyl ether for extraction from whole fish homogenates. The extract from any of these procedures is then methylated by treatment with diazomethane and analyzed with GC-FID (Oikari et al. 1984b). Oikari et al. (1980) reported "complete extraction" by their method.

2. SOURCES AND FATE IN THE ENVIRONMENT

2.1 SOURCES

Resins are a natural component of coniferous wood. Hence, resin acids may be released from any facet of the forestry industry; but by far the dominant source is pulp and paper-making. McLeay & Assoc. (1986) briefly describe the different pulping methods, and the range of concentrations of resin acids typical of each (Table 1). The range of concentrations is remarkably wide, ranging from undetectable to 10,000 ppb.

Resin acid loads in effluents depend upon (at least) the mill process, (Kraft, sulphite, mechanical, thermomechanical, thermochemical-mechanical), mill operation (i.e. at or below capacity, kinds of pulp being produced, wastewater recycling) and tree species. The last is the most important factor determining resin acid loads (Walden and Howard 1981) and considerable variation is possible even within the same species from different geographical areas (McKague et al. 1977).

Pine trees (*Pinus spp.*) have the highest resin acid content of Canadian softwoods, and hence produce the most toxic effluent. Resin acid content of lodgepole pine (*Pinus contorta*), based on oven-dry mass of wood chips, is 0.33% (Leach and Thakore 1976). Firs, such as alpine or balsam fir (*Abies spp.*) and Douglas-fir (*Pseudotsuga menziesii*) produce lower levels of resin acids, as do various species of spruce (*Picea spp.*). White spruce (*P. glauca*) has a resin acid content of only 0.12%, while alpine fir (*A. lasiocarpa*) is much lower again, at 0.03%. Comparatively low resin acids contents are found in western hemlock (*Tsuga heterophylla*) and western red cedar (*Thuja plicata*).

As a consequence of this wide variation in resin acid content, the dominant species in mill furnish may not necessarily be the dominant source of resin acids in effluents. Leach and Thakore (1976) describe a B.C. mill in which lodgepole pine contributed only 15-25% of total mill furnish, but provided 40% of the resin acids. That mill produced, on average, about 0.55 kg total effluent resin acids per tonne of pulp.

Resin acid content of wood chips declines with age (i.e. length of time in storage; Rogers et al. 1971) and also differs between sapwood and heartwood of the individual tree (Leach and Thakore 1976). Highest resin acid contents are in the bark itself, since they function as a defence mechanism against wood-boring insects. Rosehart et al. (1974) found that fingerling rainbow trout survived indefinitely in a 1:30 (w/w) solution of water and ground heart wood extract from spruce or poplar, but 1:30 solutions of bark extract were toxic, with median survival times of 9 h (poplar) to 1 h (white spruce). They did not measure resin acid concentrations.

TABLE 1: RANGE OF CONCENTRATIONS (UG/L) OF RESIN ACIDS IN UNTREATED AND BIOTREATED WHOLE MILL EFFLUENTS DERIVED FROM VARIOUS PROCESSES[a].

RESIN ACID	PROCESS						
	UKME	BKME	USME	BSME	GDWD	(C)TMP[b]	PAPER
ABIETIC[c]	30-9970	<20-4800	520-4840	<10-1000	210-16000	4210	50-1900
ABIETIC[d]	<20-3630	<10-1780	437-500	<10-100	14-4200	8860	50
DHA[c]	990-5780	30-4580	700-4620	<20-8500	490-15100	5330	227-4000
DHA[d]	<20-1930	<1-2140	247-1100	10-700	8-5800	15370	1000-3900
ISOPIMARIC[c]	70-4120	<20-4800	100-5070	<10-300	150-9300	2410	30-1200
ISOPIMARIC[d]	<20-1420	<10-930	100-294	<10-310	12-7900	6940	780-800
L-PIMARIC[c]	<10-2700	<10-2400	100-510	<10-100	80-22000		12
L-PIMARIC[d]	<10-30	<1-1190	200	<10-60	11-1800		
NEOABIETIC[c]	<50-1200	<10-1000			30-6800		29-450
NEOABIETIC[d]		<1-150			<1-3800		
PALUSTRIC[c]		90-100			300-7700		63
PALUSTRIC[d]		80					
PIMARIC[c]	100-1830	<20-1010	490-1140	<20-30	20-6800	2230	<20-610
PIMARIC[d]	<20-890	14-540	20	<20	<1-5700	4350	500-800
SANPIMARIC[c]						650	9-275
SANPIMARIC[d]						1830	45-110

(a) u/b kme - unbleached/bleached kraft mill effluent
u/b sme - unbleached/bleached sulphite mill effluent
gdwd - groundwood, mechanical pulping
(c)tmp - (chemi) thermomechanical pulping
(b) Servizi and Gordon 1986
(c) untreated effluent, may or may not be clarified
(d) biotreated effluent

Resin acids are released during the bark removal stage of wood preparation and during the pulping stage itself, when the wood is chemically or mechanically softened and lignin is removed. McKague et al. (1977) found seven resin acids (abietic, palustric, dehydroabietic, neoabietic, isopimaric, pimaric and sandaracopimaric) were responsible for most of the fish toxicity of woodroom (bark removal) effluents when softwood species were being used. Again, hardwoods produced woodroom effluents much lower in resin acids.

Since resin acids arise from the wood furnish, the other stages of pulp production (e.g. bleaching, brightening, paper making) do not contribute to resin acid discharge (although bleaching promotes the formation of chlorinated forms) (Leach & Thakore 1975). Pulping itself usually releases most of the resin acids from a pulp plant; the different processes used inevitably lead to wide differences in resin acid loads. McLeay and Assoc. (1986) have discussed this in detail.

For kraft mills, resin acids concentrations vary widely (Table 1). Concentrations in final effluents tend to be less from bleached pulp than from unbleached because of dilution from the very large volumes of water used in bleaching (90 to 130 x 106 L/d from a kraft mill of average capacity (Kovacs 1986), but of course the total loading is the same.

Biological treatment of wastes (trickling filters or aerated lagoons) is very effective at degrading resin acids, usually reducing concentrations by at least 90% and as much as 99% (McLeay & Assoc. 1986). In Canada, there are presently 30 bleached kraft mills discharging into fresh water (9 in Ontario) of which 17 (3 in Ontario) have secondary treatment (aerated lagoons) of mill effluents (Kovacs 1986).

Sulphite mills produce resin acids in about the same proportions as kraft mills, although the concentrations may be somewhat lower. Abietic acid is the most abundant, followed by dehydroabietic, isopimaric, and pimaric acids. Levopimaric acid may also be present, but is considered of minor consequence (Walden and Howard 1981). Generalizations about actual effluent concentrations are difficult because the ranges are so wide. Rosehart et al. (1974) showed that toxicity of effluent from sulphite mills was independent of base used (Na, Mg or Ca), although they did not measure resin acid content. High-yield plants produced less toxins than low-yield plants, because more acids remain in the pulp, but tree species was the most important variable.

Very high resin acid concentrations can be present in effluents from mechanical pulping operations (Table 1; Leach and Thakore 1976). Mechanical pulping does not delignify the pulp to any great extent, but wood extractives, including resin acids, are still removed. Because there are no chemical baths for the pulp, water use in mechanical pulping is (relatively) small, and resin acids are much less diluted than from kraft or sulphite mills. Again, the total load of resin acids (output per day) will depend more upon the amount and species of wood furnish than the pulping process. Nevertheless, perhaps because other constituents are not released by mechanical pulping in the same quantities as by other methods,resin

acids are of particular importance in the effluent of mechancial pulping mills, accounting for 60-90% of total effluent toxicity (Leach and Thakore 1976).

Even though concentrations of resin acids are higher in mechanical pulping, this process probably produces less resin acid loading per kilogram of wood processed than kraft or sulphite mills, because some of the resins would not be solubilized without chemical treatment (McLeay & Assoc. 1986). Solubilization of resins from thermomechanical (TMP) or chemithermo-chemical (CTMP) mills would be expected to be greater than from strictly mechanical mills, because TMP and CTMP use heat, or heat plus chemicals, to soften the wood chips (McLeay & Assoc. 1986). Limited data from a B.C. mill alternating between TMP and CTMP pulping (Servizi and Gordon 1986) indicate sharply higher resin acid production from the latter process (Table 1) as would be expected from the more severe extraction conditions.

Paper plants also release resin acids, but as these are so often integrated with pulp mills in Canada, and practise internal recycling of waste waters, it is difficult to determine what part of the effluent load is due to paper-making (McLeay & Assoc. 1986). The data available indicate that all the resin acids in Table 1, except palustric, are found in paper-making waste water (palustric acid was probably present but not identified). Abietic and dehydroabietic acids quantitatively dominate resin acids in paper-making effluents (McLeay & Assoc. 1986).

Pulp and paper mills are not the sole source of resin acids to surface waters. Areas where logs are stored, boomed, or floated downstream may also receive some resin acid contamination. Fox (1976, 1977) reported concentrations of dehydroabietic acid up to 15 ug/L, and averaging 5 ug/L, in the Nipigon River, which empties into Lake Superior. There are no pulp mills along this river, but for many years it was used to float logs to a mill on the lake. Leaching of resin acids from sunken logs accumulated on the bed of the river was seen as the probable source of dehydroabietic acid. No dehydroabietic acid was detected in Jackfish River nearby, a small river not used for log drives.

2.2 PERSISTANCE AND DEGRADATION

Most of the pertinent literature on persistence of resin acids has been summarized by McLeay & Assoc. (1986: Section 1.3); that literature is re-examined here only to explore any general trends that may be discernable.

Hemingway & Greaves (1973) demonstrated that resin acids are biodegraded in the laboratory by microflora from river water samples. Servizi et al. (1986) used acute lethality bioassays with a salmonid and daphnid species to show that microbial transformation products of resin acids were over ten times less toxic than the parent compounds. Pulp mill biological treatment processes are also effective for degrading resin acids and removing acute lethality as measured by fish toxicity tests (Servizi and Gordon, 1986).

Aerated lagoons with retention times of 3-5 days are effective at reducing resin acid concentrations by 90% or more (McLeay & Assoc. 1986). In a bench-scale aerated lagoon, total resin acid content of kraft mill effluent was reduced, on average, 72, 79 and 94% after 29, 58, or 99 hours of retention, respectively (Rogers et al. 1975).

Decomposition of resin acids apparently requires bacterial or fungal enzyme systems (biodegradation); that is, the compounds are stable against abiotic breakdown (Levinson et al. 1968). Ability to degrade the acids is not universal among microbes, but it is not rare either. For example, Hemingway and Greaves (1973) tested 69 bacterial isolates from wood sources for their ability to utilize sodium salts of mixtures of resin acids. Few of the isolates could use resin acids as their major carbon source, but 11 species did show some potential to degrade the compounds: six species of *Bacillus, Eschericia coli, Flavobacterium sp., Pseudomonas sp.* and two unidentified.

Kutney et al. (1981a) screened 17 fungal isolates, and 12 bacterial isolates, including unidentified fungi and bacteria isolated from kraft mill effluent, for their ability to degrade high concentrations (40 mg/l) of dehydroabietic acid (DHA). Twelve fungi, mostly from the fungi imperfecti, were found to have some capability to degrade DHA, as were nine bacteria (*Bacillus sp., Pseudomonas spp.*, and several unidentified). In short-term incubation (24-96 hours), one fungus, *Mortierella isabellina*, (a zygomycete) was far more effective than the others, biodegrading 98% of the DHA.

In a smaller test using isopimaric acid, *M. isabellina* was the only organism among five fungi (one a yeast) and six bacterial isolates (two *Pseudomonas* and four unidentified) to show significant detoxifying ability. At the other extreme, two species of bacteria (*Flavobacterium resinovorum*, and *Alcaligenes eutrophus*) have recently been identified that are able to subsist on resin as their sole carbon and energy source (Biellmann et al. 1973a, b). *M. isabellina* and other fungi must be supplied with an additional source of energy for resin acid detoxification to proceed (Kutney et al. 1985).

Hemingway and Greaves (1973) found that even though they could detect no reduction in DHA concentrations in cultures containing single species of bacteria, which in screening tests had shown some ability to degrade DHA, samples for water from two rivers, and sewage sludge, rapidly reduced concentrations below the detection limit (20 h at 27°C). It is likely that biodegradation in nature normally is accomplished by a consortium of species rather than by one or a few species alone.

The biochemical routes of detoxification and decomposition of several resin acids are known in some detail. Evidently bacteria degrade the acids along quite different routes than do fungi. Studies with *Mortierella isabellina* (Kutney et al. 1981a, 1985), demonstrate that this species detoxifies dehydroabietic acid by first adding a hydroxyl group to C-2 on the first ring (A), followed by hydroxylations at C-15 and C-16 on the methyl end of the molecule (Figure 1). The last two steps are apparently carried out by "mature" cultures, i.e., those in stationary phase.

There is no ring cleavage, nor any further degradation after the trihydroxyl form, but these small changes are sufficient to render the molecule nonacutely toxic to fish or *Daphnia* at typical environmental concentrations (Servizi et al. 1986).

The detoxification of isopimaric acid is identical except that the dihydroxylation at C-15 and C-16 removes a double bond (Kutney et al. 1981b); a similar sequence has been deduced for abietic acid (Kutney et al. 1982). Again, despite the relatively small changes in structure, the hydroxylated metabolites of isopimaric and abietic acid are non-acutely toxic to sockeye salmon or *Daphnia* at concentrations as high as 60-100 mg/L (Servizi et al. 1986).

In contrast to the above, degradation of resin acids by bacteria is complete, and even in the early stages follows a different route than that used by *M. isabellina* (Biellmann et al.1973a, b). When solutions of mixed resin acids labelled with $14^{\circ}C$ were inoculated with river water (containing a natural microbial population), the CO_2 released during the period of rapid decay was radioactive, indicating that decomposition of the resin acids was complete. Of the total added radioactivity, 80% was released in CO_2 after 90 h (Hemingway and Greaves 1973).

Decomposition of dehydroabietic acid by *Flavobacterium resinovorum* begins with an oxidation of C-3 (Ring A) and C-7 (Ring B), followed by a dihydroxylation of the aromatic ring at C-11 and C-12 (Figure 1). The aromatic ring is then cleaved, to produce 2-isopropyl malic acid and two other single-ring acids (Biellmann et al. 1973a). Alternatively, Rings A and B may remain together as a triketone after removal of the aromatic ring; a series of reactions then opens these rings and produces simple acids that may be completely metabolized by mainstream pathways (Biellmann et al. 1973a). The other species investigated, *Alcaligenes eutrophus* and *Pseudomonas sp.* use similar pathways, i.e. oxidation at C-7, dihydroxylation of the aromatic ring, removal of the aromatic ring (leaving a diketone), and cleavage of ring B (Biellman et al. 1973b). The enzyme battery needed for this complex sequence of reactions is apparently not present in fungi; the first intermediate product, 7-oxodehydro- abietic acid, is sometimes reported from kraft mill effluent and receiving waters (Brownlee and Strachan 1977), which suggests that bacterial degradation occurs in nature.

The difference between fungal and bacterial pathways is also shown by their response to acidity. *M. isabellina* grown on potato dextrose agar depresses the medium's pH when growth is in log phase. When abietic, dehydroabietic, or isopimaric acids are added, the pH depression is augmented, dropping as low as 3.5 for cultures started at neutrality (Kutney et al. 1981a,b 1982). However mixed inocula (river water) exposed to resin acid mixtures at various initial pH levels did not degrade the acids at all until the pH had been raised metabolically to 7.3 (Hemingway and Greaves 1973). The pH depression associated with growth of *M. isabellina* is a normal effect of its metabolism and would not be significant in the dilute freshwater environments outside the laboratory (Kutney et al. 1982). However it shows how bacterial and fungal decomposition may differ in response to environmental factors.

Dehydroabietic acid (DHA) is likely to be more persistent than the other resin acids, because it has one complete benzene ring, which is more resistant to enzymatic attack, and thus microbial decomposition (Brownlee et al. 1977). That feature, plus the fact that DHA is often produced in large quantities, results in it being the most commonly found resin acid in receiving waters.

This is exemplified by studies done on Nipigon Bay, Lake Superior, which receives effluent from a kraft pulp mill (Fox 1976, 1977, Brownlee and Strachan 1977, Brownlee et al. 1977). In the plume of warm effluent extending into Nipigon Bay, dehydroabietic acid persisted longer than any of the other organic compounds considered, and was still present at detectable levels 2 km from the outfall (Fox 1976, 1977). Outfall concentrations of DHA were 1100 to 9000 ug/L, and at 2 km, concentations of 10 to 35 ug/L were typical. Furthermore, DHA could be detected, albeit at low levels, in water outside the mill effluent plume, while the other compounds could not (Fox 1976).

A comparison of the decline of DHA concentrations in water with those of sodium, a conservative tracer, showed a close match. Hence, simple dilution, as opposed to microbial degradation, was suggested as the most important short-term removal mechanism for DHA (Fox 1976, 1977). Faster removal rates, implying biotic action, have been observed in Finnish lakes (Oikari et al. 1980). However, pulp mill loadings to Finnish lakes tend to be high, compared to Canada, and flushing rates low (McLeay & Assoc. 1986), which may result in a larger and more efficient microbial community (because they can operate on higher concentrations for longer times). Volatility of DHA is low (Fox 1976), so diffusive transport from water to air is unlikely to be a major pathway of removal.

In the Nipigon Bay studies, sandaracopimaric acid was the only resin acid other than DHA to persist a significant distance (1 km) from the mill outfall. Abietic acid was never found except in the undiluted effluent (Brownlee and Strachan 1977). This contrasts with dehydroabietic acid, which was found over an area of 25 km^2; water concentrations exceeding the 96-h LC50 for salmonids (section 3.1) occurred in the effluent plume at distances up to 0.5 km from the mill; concentrations down to 1% of the acutely toxic levels occured at distances up to 1.7 km from the mill.

Fox (1976) observed a track of DHA-laden sediment extending some 15 km from the mill outfall; sediment concentrations of DHA were as high as 100 ug/g near the mill, and 15 km distant they were still nearly 10 times greater than the background level of 2 ug/g. Brownlee and Strachan (1977) reported concentrations of DHA in suspended sediment and bottom sediments which were over an order of magnitude higher than levels in water. A large difference in DHA concentrations between aqueous and non-aqueous phases indicates that DHA exhibits hydrophobic partitioning behavior in aquatic systems.

Using loading rates, sedimentation rates, and the depth profile of DHA in the sediments, Brownlee et al. (1977) computed a half-life for sediment-bound DHA of 21 years (in contrast, DHA half-life in the water was estimated at six weeks) and a total sediment load of 12,000 tons over an area of 64 km^2. This suggests that

a significant mass fraction of the DHA in effluents sorbs to non-aqueous phases such as suspended particles and organic matter and ultimately accumulates in bottom sediments. Pimaric and abietic acids were also present in the sediments, but in unquantified amounts.

Although microbial action in the water column can be effective in terms of the fraction of aqueous phase DHA in a discharge which is biodegraded, the Nipigon Bay studies suggest that water phase fate processes do not protect the receiver from substantial long-term sediment contamination. DHA half-life in the receiver depends primarily on half-life in sediments because this is where most of the substance is located. DHA is, therefore, quite persistent in aquatic environments.

3. EFFECTS OF RESIN ACIDS ON FISH

3.1 ACUTE TOXICITY

Bioassays of acute lethal toxicity of resin acids to fish are summarized in Table 2; the acids are arranged approximately in ascending order of toxicity, i.e. from the greatest to the least LC50. With the exception of dehydroabietic acid and results from Servizi et al. 1986, all of the salmonid bioassays have been carried out in the laboratories of B.C. Research in Vancouver, using standard bioassay techniques to obtain reproducible data.

The data of Davis and Hoos (1975) arise from an interlaboratory comparison using one source of dehydroabietic acid and a standard bioassay procedure. Six laboratories, both government and private, were involved. With the exception of one outlying LC50 value for trout (1.7 mg/L), the data are roughly comparable (Table 2).

For comparative purposes, data on resin acid acute toxicity to fathead minnow (*Pimephales promelas*) from a standard protocol (USEPA, 1987) are included. The rank order of resin acid LC50's from this standard test agree well with the ranking for salmonids, but fathead minnows are slightly less sensitive. The following discussion pertains to salmonid species.

Rogers et al. (1975) and Kruzynski (1979) have described the sequence of symptoms preceeding death of sockeye salmon (*Oncorhynchus nerka*) exposed to dehydroabietic acid in flow- through bioassays. Healthy fish maintained a constant position against the current, and schooled beneath whatever cover was provided. Disturbance caused the school to tighten and retreat further beneath cover. About 20 h after exposure began, schooling behaviour began to weaken, and response to disturbance was reduced and eventually eliminated. Fish appeared to develop progressive loss of muscle co-ordination and consequently lost the ability to maintain their position, and so were swept around the tank by the current. Final symptoms, about 3 h after equilibrium loss, were weakness, passive inverted drifting around the tank, muscle spasms and death (Kruzynski 1979, Rogers et al. 1975). This set of behavioral changes are very similar to the set of clinical signs or syndrome described by McKim et al. (1987) which indicates a narcotic action in fish acute toxicity.

Dehydroabietic acid is the least toxic resin acid, and isopimaric and sandaracopimaric the most toxic, although the latter's LC50, being based on coho salmon (*Oncorhynchus kisutch*), is not strictly comparable to those based on rainbow trout (*Salmo gairdneri*). The most striking feature of the data in Table 2 is the narrowness of the range of LC50 for all eight acids: the 96 h LC50 for any resin acid at circumneutral pH may safely be predicted to lie between 0.5 and 1.5 mg/L for

TABLE 2: ACUTE TOXICITY (96h-LC50 IN MG/L) OF RESIN ACIDS TO FISH ALL BIOASSAYS STATIC UNLESS INDICATED OTHERWISE

Compound	Species	LC50	Test Conditions	Reference
Dehydroabietic acid	Rainbow Trout	1.0	pH 7.0, hardness 23.0 mg/L ($CaCO_3$)	Davis and Hoos 1975
	Rainbow Trout	1.1	pH 7.0, hardness 47.0 mg/L	Davis and Hoos 1975
	Rainbow Trout	1.2	pH 7.0, hardness 5-6 mg/L	Davis and Hoos 1975
	Rainbow Trout	1.7	pH 6.9, hardness 5.0 mg/L	Davis and Hoos 1975
	Rainbow Trout	1.2	pH 9.0, hardness 4.0, Temp. 9.3°C	Davis and Hoos 1975
	Rainbow Trout	1.3	pH 7.0, hardness 10.0 mg/L	Davis and Hoos 1975
	Rainbow Trout	1.1	pH 6.9-7.1	Leach and Thakore 1976
	Rainbow Trout	0.8-1.1	pH 6.4-6.5, hardness 4-7	B.C.Research 1977b
	Rainbow Trout	0.8-1.2	pH 6.4, hardness 4.8 mg/L	Chung et al. 1979
	Rainbow Trout	0.77	Semi-static, pH 6.5, hardness 5.0 mg/L	McLeay 1976
	Sockeye Salmon	1.4	pH 7.1, hardness 47.0 mg/L	Davis and Hoos 1975
	Sockeye Salmon	2.1	pH 7.7, hardness 85.0 mg/L	Davis and Hoos 1975
	Sockeye Salmon	0.5	Flowthrough, pH not given	Rogers et al. 1975

Table 2 (continued)

Compound	Species	LC50	Test Conditions	Reference
	Sockeye Salmon	2.1	pH 7.2-8.0, hardness 85 mg/L	Servizi et al. 1986
	Sockeye Salmon	0.5	Flowthrough, pH 6.3-6.4, hardness 49 mg/L	Kruzynski 1979
	Sockeye Salmon	0.79	Flowthrough, pH 7.0-7.1, hardness 49 mg/L	Kruzynski 1979
	Sockeye Salmon	0.88	Flowthrough, pH 6.8-6.9, hardness 49 mg/L	Kruzynski 1979
	Coho Salmon	1.4	pH 7.0, hardness 7.0 mg/L	Davis and Hoos 1975
	Coho Salmon	1.8	pH 7.1, hardness 5-6 mg/L	Davis and Hoos 1975
	Coho Salmon	0.75	semi-static pH 7.0	Leach and Thakore 1977
	Fathead Minnow	2.10	pH 7.7, hardness 45 mg/L	USEPA 1987
Pimaric acid	Rainbow Trout	0.8	pH 6.9-7.1	Leach and Thakore 1976
	Rainbow Trout	0.7-1.2	pH 6.4, hardness 4.8 mg/L	Chung et al. 1979
	Rainbow Trout	0.8	pH 6.4-6.5, hardness 4-7 mg/L	B.C.Research 977b, 1978
	Coho Salmon	0.32	semi-static, pH 7.0	Leach and Thakore 1977

Table 2 (continued)

Compound	Species	LC50	Test Conditions	Reference
Abietic Acid	Rainbow Trout	0.7	pH 6.9-7.1	Leach and Thakore 1976
	Rainbow Trout	0.7-1.5	pH 6.4, hardness 4.8 mg/L	Chung et al. 1979
	Rainbow Trout	0.9	pH 6.4-6.5, hardness 4-7 mg/L	B.C. Research 1977b, 1978
	Coho Salmon	0.41	semi-static, pH 7.0	Leach and Thakore 1977
	Sockeye Salmon	0.2	pH 7.2-8.0	Servizi et al. 1986
	Fathead Minnow	2.38	pH 7.5, hardness 46 mg/L	USEPA 1987
Levopimaric acid	Rainbow Trout	0.7-1.0	pH 6.4, hardness 4.8 mg/L	Chung et al. 1979
	Rainbow Trout	0.6	pH 6.4-6.5, hardness 4-7 mg/L	B.C.Research 1977b, 1978
Neoabietic acid	Rainbow Trout	0.6-0.7		Leach and Chung 1980
	Fathead Minnow	1.3-1.7	pH 7.5, hardness 44 mg/L	USEPA 1987
Palustric acid	Rainbow Trout	0.5	pH 6.9-7.1	Leach and Thakore 1976
	Rainbow Trout	0.5-0.6	pH 6.4, hardness 4.8 mg/L	Chung et al. 1979
	Rainbow Trout	0.55	pH 7.0	Leach and Thakore 1977

Table 2 (continued)

Compound	Species	LC50	Test Conditions	Reference
Isopimaric acid	Rainbow Trout	0.4	pH 6.9-7.1	Leach and Takore 1976
	Rainbow trout	0.4-1.0	pH 6.4, hardness 4.8 mg/L	Chung et al. 1979
	Rainbow Trout	0.5	pH 6.4-6.5, hardness 4-7 mg/L	B.C. Research 1977b, 1978
	Coho Salmon	0.22	semi-static, pH 7.0	Leach and Thakore 1977
	Sockeye Salmon	0.7	pH 7.2-8.0	Servizi et al. 1986
Sandaracopimaric acid	Coho Salmon	0.36	semi-static, pH 7.0	Leach and Thakore

rainbow trout. Small differences between experiments for any one acid can be attributed to differences in pH (see below).

There are several ramifications of this uniformity of toxicities. First, the toxicity of the resin acid component of pulp mill effluent will depend mostly upon the concentration of resin acids it contains rather than the toxicity of each individual form. Thus, dehydroabietic, pimaric and abietic acids, which have the highest LC50 values (Table 2), are still important toxicants in many effluents because they are present in high concentrations. Second, it may prove feasible to measure and regulate resin acids in pulp mill effluent on the basis of total resin acid content. These points are explored in detail in Section 5.

LC50 values from flowthrough or semi-static tests (in which water is changed at intervals) are consistently less than from static tests (Table 2). For sockeye salmon, Rogers et al. (1975) and Kruzynski (1979) obtained LC50 values less than half those obtained for the same species, or closely related coho salmon, with static bioassays (Davis and Hoos 1975, Servizi et al. 1986).

Similarly, Leach and Thakore (1977) found that solution-replacement assays predicted LC50 values for coho salmon of 0.2-0.75 mg/L, while nominal LC50 values from static assays were 0.6-1.6 mg/L. These values for coho salmon are again less than half those reported from static bioassay by Davis and Hoos (1975) (Table 1). For the other resin acids, LC50's from semi-static assays on coho salmon are substantially lower than those from static assays on trout (Table 2).

Rogers et al. (1975) suggested this difference in apparent toxicity is due to the greater accuracy of the flowthrough method, but they did not report pH, so the possibility cannot be excluded that, in this instance, pH was responsible for some of the difference.

However, in a set of carefully designed experiments with sockeye salmon, Kruzynski (1979) has demonstrated that static bioassays consistently underestimate the toxicity (overestimate the LC50) of dehydroabietic acid. The actual concentration of DHA to which fish were exposed was less than the original concentration because of adsorption of the acid onto the walls of the tank, and especially by the fish themselves. At a fish density of 1.1 g/L, a nominal DHA concentration of 1.88 mg/L was reduced to 1.16 mg/L after 24 h and 0.77 mg/L after 96 h. At a fish density of 2.2 g/L, concentrations were reduced to only 0.86 and 0.29 mg/L after 24 and 96 hours respectively (Kruzynski 1979). These reductions of 40-55% in 24 h were due to intake of the toxicant by the fish, as well as sorption onto the fishes' skin and mucous layer (Rogers et al. 1975). Even in a tank without fish, DHA concentration declined 31% after 24 h, apparently due to sorption onto the tank walls (Kruzynski 1979). These losses from solution suggest that resin acids exhibit hydrophobic behvior, that is, they tend to partition from water into non-aqueous phases.

The consequences of this change in concentration for apparent toxicity were obvious. At a density of 1.1 g fish/L, all fish were dead in 54 h; at the higher density (2.2 g/L), three fish out of 10 died in 40 h and the remaining seven survived to the end of the 96-hour assay; even though all fish were originally exposed to a lethal dose (1.88 mg/L) of DHA, this was soon reduced to sub-lethal levels in the high-density tank. This result leads to the conclusion that LC50 values from static bioassays in Table 2 should be considered overestimates, and the various resin acids may be more toxic than indicated.

Acute toxicity of pulp mill wastes is strongly dependent upon pH (Walden and Howard 1981, Leach and Thakore 1974, Loch and MacLeod 1974, McLeay et al. 1979a,b), with the lowest toxicity in the range of pH 8.5-9.5 (B.C. Research 1977a, McLeay et al. 1979b). This relationship in turn has been attributed to the influence of water acidity on bioavailability and toxicity of resin acids in the effluent (Walden and Howard 1981, McLeay et al. 1979a, Leach and Thakore 1974, 1976, 1977).

McLeay et al. (1979a), in a detailed study of pH-toxicity relationships of pulp mill effluent, showed that toxicity of the effluents to rainbow trout declined as pH increased from 5.0-9.5, and that a nearly identical relationship existed for dehydroabietic acid. Solutions were least toxic at pH 9.0 (kraft mill effluent) or 9.5 (DHA) and most toxic at pH 5. At pH 10 or above, both solutions killed fish quickly, but that was due to toxicity of extreme pH itself. Further, the LT50 for trout was inversely proportional and nearly log-linearly related to the predicted percentage of un-ionized acid (as calculated from pKa values), over the range pH 5-9 (Figure 2). That is, as pH increased, ionization of resin acids increased and toxicity declined, to a minimum near pH 9, where the resin acids would be 98% ionized (McLeay et. al. 1979a). The pKa, and hence behaviour, of other resin

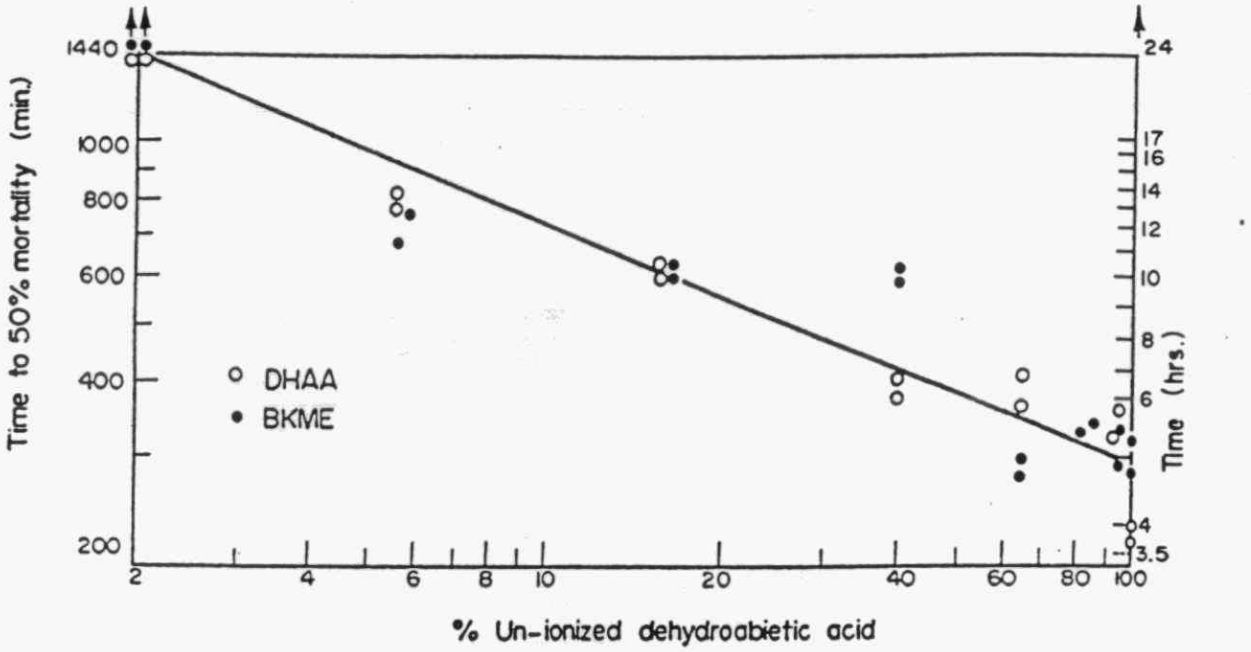

Figure 2. Toxicity of dehydroabietic acid and bleached kraft mill effluent to juvenile rainbow trout as a function percent unionized resin acid calculated for each test pH (McLeay et al. 1979a).

acids is thought to be similar to DHA (Oikari et al. 1983), which has a pKa of 7.25 (McLeay et al. 1979a).

These results have been confirmed by Zanella (1983) for fathead minnows (*Pimephales promelas*) and *Daphnia magna*. His results (Figure 3), covering the normal range of environmental pH in non-acidified surface water, illustrate toxicity declining exponentially with pH, exactly the same pattern as described by McLeay et al. (1979a). DHA is fifteen to thirty times more toxic at pH 6.5 than at pH 9 (Zanella 1983); for salmonids, a 5 mg/L solution of DHA at pH 6.4 is more toxic than a 10 mg/L solution at pH 7.5 (Leach and Thakore 1976).

McLeay et al. (1979a) argue that the pH-dependence of resin acid toxicity results from the change in relative solubility of the acids in lipid and water. As pH increases, weak acids such as these ionize and then, having a stronger polarity, become more soluble in water and less soluble in lipids. In ionic form, they are much less likely to partition across the gill membrane as readily as the more lipid-soluble and hydrophobic un-ionized form that exists in acidic water. Gill uptake of other weak acid toxicants has been predicted from their pKa and relative lipid solubility; these chemicals show the same pH-dependence of toxicity as resin acids (McLeay et al. 1979a).

There are two important ramifications of this hypothesized effect of pH. First, bioassays which do not control or at least measure pH of the diluting water are of little value. Many bioassays are now standardized to near pH 7.0. However, within the pH range 6.4-7.5, the degree of ionization of DHA, and hence its toxicity, responds to small changes in pH (Figure 4). Hence, some of the variation in LC50 values in Table 2 may be simply a consequence of pH differences.

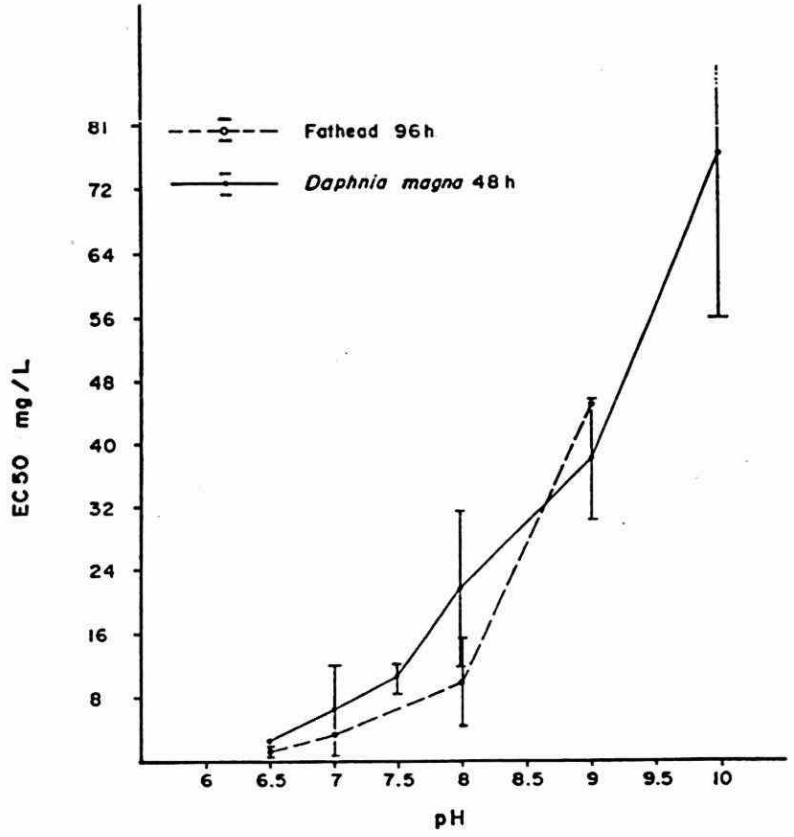

Figure 3. Toxicity of dehydroabietic acid as a function of test solution pH (Zanella 1983).

Second, any surface water criterion for resin acid loading must take into account the variation of pH in surface waters. Most pulp mill effluent is neutralized to pH near 7 (McLeay et al. 1979b, Leach and Thakore 1976), but surface waters in Ontario vary in pH from 8.5 to 5.0, or even lower where acidification has occured. Significant changes in toxicity of resin acids will occur from about pH 5.5-8.5, the entire range for Ontario surface water. Pulses of acidic water have also been associated with spring melt in some northern Ontario waters.

Water hardness and alkalinity have also been shown to alter toxicity of pulp mill effluent (Eco-Research Ltd. 1974) with toxicity decreasing linearly with increasing hardness, except at very low (10 mg/L as $CaCO_3$) or high (150 mg/L) values (McLeay et al. 1979a, Eco-Research Ltd. 1974). However, when hardness or alkalinity is changed while pH is kept constant, the effect of buffering capacity on effluent toxicity is sharply curtailed, and may not be present at all if hardness exceeds 100 mg/L as $CaCO_3$ (McLeay et al. 1979a). No attempts have been made to establish whether toxicity of resin acids alone is affected by hardness ; certainly there would be some effect from the buffering of pH variation, but the response of whole effluents suggests that the direct effect of hardness is small. For whole kraft mill effluent, McLeay et al. (1979a) have shown that, when the effect of pH is removed, virtually all the remaining variation in toxicity to trout is accounted for by hardness or alkalinity. Since resin acids are major toxicants of kraft mill effluent, this must be true for them also.

Dissolved oxygen concentration in the ambient water strongly influences toxicity of pulp mill wastes (Hicks and DeWitt 1971) and resin acids (Kruzynski 1979).

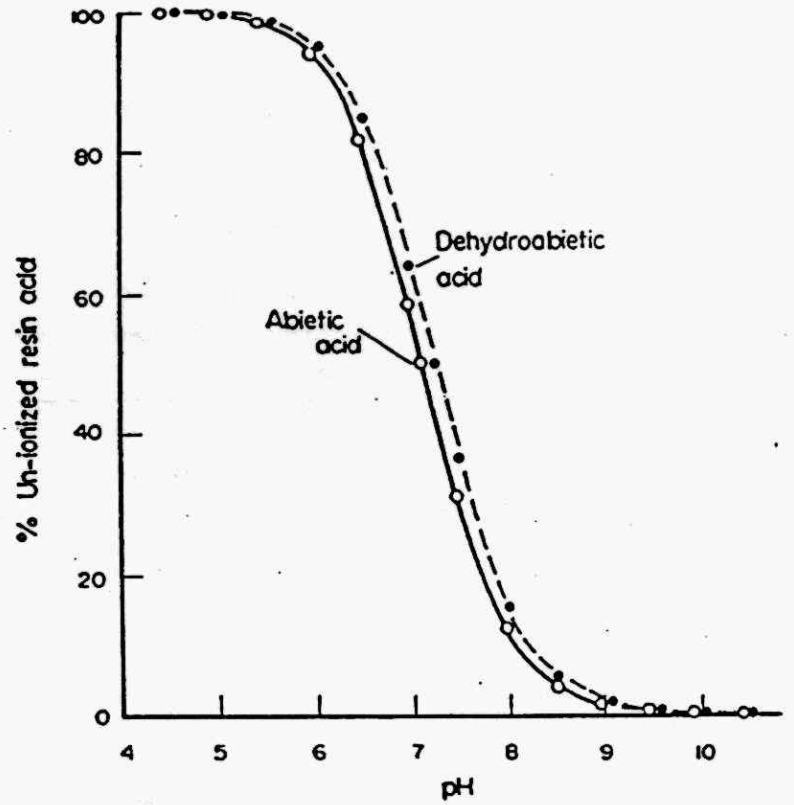

Figure 4. Percentage of total resin acid unionized in solution, calculated from resin acid pKa values for each water pH value (McLeay et al. 1979a).

Resin acids interfere with normal oxygen uptake by the gill lamellae (see Section 3.2) and hence induce oxygen starvation. Affected fish show symptoms of respiratory distress, i.e. "coughing" and increased gill ventilation. Since oxygen cannot be taken up by intoxicated fish with the usual efficiency, a low oxygen tension that normally would be tolerated may be lethal. Sockeye salmon exposed to a normally sublethal concentration of dehydroabietic acid (0.65 mg/L) at dissolved oxygen levels of 75% saturation (at 11.4°C) experienced 100% mortality, while fish exposed under identical conditions in well-oxygenated water (90% saturation) experienced only 5% mortality (Kruzynski 1979). The mortality rate in hypoxic water began to decline once fish deaths reduced oxygen consumption and raised DO level to about 85% of saturation. These observations compare well with those for whole pulp mill effluent which indicate that exposure increases the respiratory rate and susceptibility to normally non-fatal hypoxia (Hicks and DeWitt 1971).

The reciprocity of resin acid and hypoxia stresses is critical: hypoxia increases the toxicity of resin acids and resin acids increase the lethality of hypoxia. Further, impairment of oxygen transport by resin acids induces increased gill ventilation, which to some extent further accelerates resin acid uptake (Kruzynski 1979) because more contaminated water passes over the gills. Since reduced oxygen tension in receiving waters as a result of BOD of pulp mill wastes is a common problem, the above relationships are of particular significance.

For salmonids at least, resin acid toxicity is greater at high temperatures (McLeay 1976). This is seen as a consequence of an intolerable "stress load" resulting from

simultaneous effect of the toxicant and hyperthermia. Also, elevated temperatures increase the metabolic oxygen demand of the animals while lowering the saturation concentration of dissolved oxygen. The bioassays in Table 2 were carried out at 10-12°C to avoid thermal stress on the fish.

3.2 SUBLETHAL EFFECTS OF RESIN ACIDS

Although sublethal effects of whole pulp mill effluent have been extensively studied (Davis 1973, 1976), sublethal toxicity of resin acids has been examined mostly for dehydroabietic acid. Kruzynski (1979) reported a detailed examination of juvenile sockeye salmon (*Oncorhynchus nerka*) exposed to sublethal concentrations of DHA (0.65 mg/L) for 120 h at pH 6.7-7.0. The exposure caused a disruption of the water and ionic balance of the smolts, characterized by accelerated osmotic absorption of water. Concentrations of sodium and chloride ions in blood plasma decreased, as did plasma osmolality, and water content of muscle tissue increased. Blood hematocrit (volume of red blood cells per ml of blood) increased as a result of swelling by red blood cells; this, in turn, was in response to the lowered plasma osmolality.

There is evidence that this osmotic imbalance was itself a secondary effect, following hypoxic stress (lowered ability of the gill to take up oxygen) brought on by exposure to the DHA. In response to reduced respiratory efficiency, the fish increase the rate of water passage over the gill, which would prompt the hydration and loss of osmotic balance. When these fish were transferred to seawater, the added salinity stress was frequently fatal (Kruzynski 1979).

Similar, but less pronounced, results were obtained by Nikinmaa and Oikari (1982). They exposed adult female rainbow trout (mean body weight 871 g) to a 2 mg/L concentration of a mixture of eight resin acids, dominated by abietic and palustric acids, at a pH of 7.2-7.4. There was a significant decrease in plasma chloride concentrations after 24 h. However, there were no changes in levels of sodium or potassium; in all fish but one, chloride returned to normal levels within 24 h after exposure to the acids was stopped.

On the other hand, Oikari et al. (1983) found no changes in levels of blood plasma ions in 1-year-old rainbow trout exposed to 0.8-2.0 mg/L DHA (mean 1.2 mg/L) for four days (the exposure caused no mortality, perhaps due to the large size of the fish). Concentrations of plasma Na^+,Cl^-, K^+, Mg^{2+} and Ca^{2+} in exposed fish were not significantly different from controls, nor was there any hydration of muscle or liver tissue. They concluded that there was no effect of DHA on osmoregulation or ionic balance in these fish, the reverse of Kruzynski's (1979) conclusion.

The absence of this effect was confirmed in the field by caging 2 year-old fish at various distances from a kraft mill outfall (Oikari et al. 1985b). Even the fish kept < 1 km from the mill effluent (mean total resin acid content of water: 12-21 ug/L, pH 6.7-6.9) exhibited no serious or consistent changes in plasma ion concentra-

tions, except for an increase in sodium, attributable to the high Na^+ concentration of kraft mill effluent (Oikari et al. 1985b).

It is possible that the severity of osmotic effects of DHA on sockeye salmon observed by Kruzynski (1979) is specific to this and other anadromous species. Kruzynski was interested particularly in pulp mill effluent effects on young salmon making their first migration to the sea. The ionic and osmotic regulatory systems of salmon smolts undergo a profound rearrangement when the fish enters seawater and the external medium switches from hypotonic to hypertonic. Hence, the osmoregulation system of salmon smolts at the time of their seaward migration is labile, and much more sensitive to external conditions than in strictly freshwater fish.

Acute exposure of rainbow trout to DHA did produce a range of other physiological responses, especially in the blood and liver (Oikari et al. 1983). Haematocrit (packed cell volume of erythrocytes), haemoglobin concentration and concentration of plasma proteins increased compared to control fish by 31-44%, indicating a fluid efflux from the blood. There was also a marked decrease in liver mass which, since water content was unchanged, suggests that energy reserves (glycogen) were exhausted.

A parallel increase in concentration of the enzyme aspartate aminotransferase also indicates a switch to amino acids as fuel. Activity of lactate dehydrogenase in white muscle was significantly increased, and there was a shift in the proportion of isozymes to a form more efficient under conditions of hypoxia. Finally, inhibition of certain enzymes in the liver and kidneys reflected a sharply decreased ability to eliminate DHA or other poisons. Other major organs studied (spleen, stomach, heart) and all other enzymes examined were unaffected.

For coho salmon, differential mortality and physiological effects from DHA exposure have been associated with exercise levels of the fish (Iwama et al. 1976). Juvenile salmon were exposed to 0.75 mg/L DHA (pH 6.8-6.9) in recirculating tanks (which force the fish to swim to maintain position) for 48 hours. By the end of the exposure period, six out of 64 fish in the "low exercise" treatment had died, and survivors in low and intermediate treatments exhibited sluggish swimming and disorientation. At the low exercise level, swimming co-ordination was poor and the fish lost the fright response to shadows or sudden movement. These behavourial symptoms are very similar to those described by Kruzynski (1979) and Rogers et al. (1975) for sockeye salmon exposed to acutely lethal doses of DHA. Fish in the high exercise treatment exhibited neither mortality nor unusual behaviour.

Mean clotting time increased in DHA-exposed fish, and total white blood cell count decreased. Again, these responses were most pronounced in the "low exercise" treatment and not present in the "high exercise" treatment (Iwama et al. 1976). The lowered white blood cell count is believed to be part of a general stress response, mediated by secretion of corticosteroids; increased clotting time may reflect decreased levels of thrombocytes, a blood cell type important in clot formation. That change is also part of the corticosteroid-mediated stress response, but whether it was operating in this instance was not determined.

However, a marked increase in corticosteroids has been demonstrated within a few hours of exposure of yearling sockeye salmon to lethal or near-lethal concentrations of DHA (Dye and Donaldson 1974).

There were no changes in haematocrit, erythrocyte count, or erythrocyte sedimentation rates however, in response to the DHA at any exercise level. This contradicts the results of Oikari et al. (1983) and Kruzynski (1979; see Section 3.1) that plasma hypoxia is a key symptom of DHA poisoning. On the other hand, forcing the fish to exercise may have ameliorated this response because the increased volume of water passing over the gills during rapid swimming could compensate for the decline in oxygen capture efficiency. Moreover, the current apparently enhanced oxygen uptake sufficiently to compensate for the faster rate of DHA absorption expected for the same reason. Even in the low exercise level, water was flowing by the fish at a rate of 0.5 body lengths per second (high exercise = 1.97 body lengths/s), much faster than in a normal flow-through bioassay. Hence, the disparate results are reconcilable; but no experimental proof of this effect is available.

Plasma hypoxia or related respiratory changes are commonly observed in salmonids acutely exposed to resin acids (Nikinmaa and Oikari 1982) or whole kraft mill effluent (Davis 1973, Oikari et al. 1985a, b). For example, 24-h exposure of juvenile rainbow trout to 2 mg/L mixed resin acids (pH 7.4) lowered arterial oxygen tension from 96 to <75 mm Hg (Nikinmaa and Oikari 1982). The initial cause apparently is swelling of epithelial tissue on the secondary gill lamellae (Tuurala and Soivio 1982). In addition, secretion of excess mucus onto the gills has been observed in response to bleached whole kraft effluent (Davis 1973), but whether resin acids were responsible is not known. This epithelial swelling increases the diffusion distance from surrounding water to capillaries and markedly reduces oxygen uptake. Simultaneous with this, there is substantial vasoconstriction of secondary lamellar capillaries and an increase in haematocrit of lamella blood (Tuurala and Soivio 1982), both due to a decrease in plasma volume. In addition, resin acids may affect red blood cells directly, by decreasing erythrocyte pH and thus lowering the affinity of haemoglobin for oxygen (Nikinmaa and Oikari 1982).

Liver disfunction is another consequence of resin acid exposure. This has been shown for rainbow trout exposed to pure DHA (Oikari et al. 1983) and for resin acid mixtures (Oikari and Nakari 1982, Nikinmaa and Oikari 1982). Adult female rainbow trout exposed to 1 mg/L mixed resin acids for 24 h at pH 7.2-7.4 exhibited a sharp increase in plasma concentrations of bilirubin, a toxic breakdown product of haemoglobin usually detoxified by the liver. Concentrations of bilirubin increased to 300% of control fish levels during the 24 h exposure, and surprisingly continued to increase for at least 48 h after the fish were transferred to clean water, indicating a persistent disfunction of the liver (Nikinmaa and Oikari 1982). Bilirubin accumulation is linked to inhibition of the enzyme UDP-glucoronyl transferase (UDP-GT) in the liver and kidneys. Normally, UDP-GT catalyzes the conjugation of bilirubin with glucuronic acid to form a polar, water-soluble compound, bilirubin diglucuronide, which is then excreted in the

bile.Many hydrophobic organic poisons are conjugated and eliminated the same way (Martin et al. 1983).

Acute exposure to DHA inhibited UDP-GT activity in trout by up to 60% (Oikari et al. 1983), and similar inhibition was produced by resin acid-fatty acid mixtures (Oikari and Nakari 1982) and simulated kraft mill effluent (Oikari et al. 1984b). The unconjugated bilirubin accumulates in blood, producing jaundice (Kruzynski 1979; Oikari et al. 1984b).

Severe depletion of liver glycogen stores (67 to 100%) in 3-4 days have also been reported after exposure of rainbow trout to DHA (Oikari et al. 1983) and resin-acid fatty acid mixtures (Oikari and Nakari 1982). A decline of liver mass results, unless it is compensated by water uptake (Oikari et al. 1984b).

Finnish researchers have conducted studies of pulp mill resin acid toxicity in the field. Immature rainbow trout were kept in cages at various distances, 0.7-11 km downstream of a kraft pulp mill discharging to lake water (pH 6.4-6.9). Enzyme activity of liver UDP-GT was significantly inhibited (15-40%) compared to controls after 2-5 days exposure, at sites 0.7 and 3 km from the mill outfall. These fish had very low levels of resin acids (maximum 1.25 ppb total acids) in blood plasma, and also in bile. After ten days exposure, the UDP-GT inhibition passed, indicating that physiological adaptation to low contaminant levels is possible. Fish caged 6 km from the mill began to show significant enzyme inhibition after ten days exposure. These fish had no resin acids in plasma, but it was detectable in bile (Oikari et al. 1985b). No inhibition of acetylcholinesterase or consistent plasma cholinesterase inhibition was detected; the authors concluded that resin acids or other components of pulp mill effluents did not affect this aspect of neurotransmission.

Caged trout just below (0.7 km) the pulp mill outfall showed no change in blood haemoglobin levels. But, in trout exposed for ten days at 6.5-11 km from the outfall, blood haemoglobin increased dramatically, up to 43%. These results agree with results from fish exposed to DHA in laboratory tests, but it must be borne in mind that these fish were exposed to mill effluent containing fatty acids and a variety of chlorinated compounds, any of which could contribute to the effects found. Also, it is possible that fish caged near the mill compensatorily accelerated rates of both erythrocyte manufacture and destruction, so that net haemoglobin concentration did not change, while fish farther from the mill increased only the rate of erythrocyte manufacture.

Oikari et al. (1984b, 1985a) report similar results from a thirty day exposure of one-year-old trout to simulated kraft mill effluent, a mixture containing 25% resin acids, at about 0.08% of the 96-hour LC50 (approximately 50 ppb resin acids). They found no change in blood oxygen-binding capacity (haematocrit, haemoglobin, ATP in erythrocytes, plasma oxygen tension) except for an increase in magnesium content of red blood cells. However there was a sharp decline in plasma protein concentration. The increase in relative size of the spleen noticed by Oikari et al. (1983) appeared here also; it may be related to a change in rate of red blood cell production (Oikari et al. 1985b).

However, the more recent study did find subtle differences in energy metabolism of the exposed fish. Oxygen consumption was elevated; muscle lipid content increased markedly; and ammonia excretion declined. The decline in ammonia excretion, indicative of a drop in catabolism of nitrogenous compounds, is consistent with a significantly lower protein content of muscle and plasma compared to control fish. This, coupled with elevated lipid content, implies a shift from protein to lipid-based metabolism, and the greater oxygen demand of the latter in turn accounts for the rise in oxygen consumption (Oikari et al. 1985a). Thus, although there was no evidence of stress or impairment of health of the test fish, even this relatively low resin acid concentration (2 ppb) was still causing measureable changes in fish metabolism.

Very few studies of chronic resin acid toxicity have been performed. Oikari et al. (1983) exposed 2-year-old rainbow trout to 10-50 ppb DHA (mean 20 ppb; about 1-3% of 96-hour LC50) for thirty days at pH 7.2-7.3, and found effects quite different and much less than in fish acutely exposed to high concentrations. Exposed fish had substantially larger (+80%) spleens than controls, but the physiological significance of this is unclear: it may reflect storage of an expanded reserve of red blood cells. Blood parameters (haematocrit, haemoglobin concentration, leucocrit, plasma ion concentrations) were all unchanged, and energy metabolism appeared equally unaffected. Finally, no liver changes, or any abnormal enzyme activity could be detected, except for a shift in isozyme balance of lactate dehydrogenase whose significance is unclear. Summarily, the DHA concentration of 20 ppb did not seem to be causing any metabolic stress in these fish.

Other aspects of chronic toxicity of resin acids such as reproduction or growth have not been addressed. But studies with a variety of warmwater fish species in artificial streams have shown that biotreated, bleached kraft mill effluent had only minor effects on egg hatchability and growth of juveniles (NCASI 1985). Largemouth bass (*Micropterus salmoides*), bluegill sunfish (*Lepomis macrochirus*) and golden shiner (*Notemigonus crysoleucas*) spawned successfully in water containing up to 9% effluent, and 100% effluent usually had no effect on egg hatchability or posthatch survival.

For fathead minnows (*Pimephales promelas*) some retardation of growth at effluent concentrations > 10% were noted in half of the tests. Attempts to associate the extent of growth reduction with particular effluent components were unsuccessful: all of the 15 components tested, including resin acids and chlorinated resin acids, showed strong, equal correlations with the reduction in growth rate (NCASI 1985). This undoubtedly arose because the concentrations of all the components in the effluent were inter-correlated.

From the close similarity of responses to DHA (Oikari et al. 1983) and resin acid mixtures (Nikinmaa and Oikari 1982, Oikari and Nakari 1982), it seems reasonable that other resin acids will behave similarly to DHA; however, specific proof of this is needed. In addition, there is evidence that effects of resin acids on trout may be different when other components of mill effluents (in particular chlorophenols) are present (Oikari et al. 1984b).

3.3 BIOACCUMULATION

As hydrophobic substances, resin acids are likely to partition from water into the fatty tissues of fish by uptake across the gill surfaces (Kruzynski 1979). Theoretically, some bioconcentration of resin acids is expected, given their hydrophobicity as measured by octanol-water partition coefficient, Kow. For example, a log Kow of 5.8 for abietic acid has been reported (Beak Consultants Ltd. 1987).

The ionized forms of the acids, which carry a net negative charge, are relatively less soluble in lipids and thus are taken up much less readily (McLeay et al. 1979a). Estimated uptake of DHA from water into fish is about 40% greater at pH 6.5 than at pH 7.3 (Oikari et al. 1983). After entering the vascular system, lipid-soluble compounds such as resin acids are thought to bind nonspecifically to plasma lipoproteins and are then distributed throughout the body in blood (Kruzynski 1979).

Resin acids in the bloodstream are trapped and enzymatically transformed in the liver. Within the liver, the acids are conjugated with glucuronic acid, a sugar acid, to form polar, water soluble complexes. The conjugates are then transferred to the bile and eliminated by excretion into the gut (Oikari et al. 1984a, Kruzynski 1979). Nearly 100% of resin acids found in bile of trout are in the form of glucuronide conjugates; most resin acids in blood plasma are unconjugated (Oikari and Niittyla 1985). Very similar behaviour in the blood and bile has been shown for dehydroabietic, abietic, pimaric, palustric, levopimaric, isopimaric and sandaracopimaric acids, although a small proportion of abietic acid, about 16%, is conjugated to some substance other than glucuronic acid.

During acute exposure ($<$ 5 days) of trout to relatively high resin acid concentrations ($>$ 1 mg/L total acids), the ratio of concentrations of unconjugated acids in bile to that in plasma (B/P ratio) was much less than one, i.e. blood concentrations far exceeded bile concentrations. This indicates that acids may be transported by passive diffusion from blood to bile and active transport may not be necessary (Oikari et al. 1984a).

Conversely, during chronic exposure to lower acid concentrations, the B/P ratio was reversed, being much greater than one for all resin acids and as high as 10 for dehydroabietic acid. Here, the acids were travelling against a steep concentration gradient, and active transcellular transport is implicated (Oikari et al. 1984a). While the above high B/P ratios appeared in laboratory tests with pure resin acids, they do not appear to reflect the general condition. For instance, a field test in Finland with trout exposed to diluted (2-5% v/v) kraft mill effluent for thirty days revealed B/P ratios still less than one, i.e. no evidence of active transport (Oikari and Niittyla 1985). Similarly, Oikari et al. (1984b) exposed rainbow trout to simulated kraft mill effluent and found B/P ratios rarely exceeded 1.0 for any resin acid; the mean B/P ratio was 0.83 for 30-day exposures to solutions containing about 50 ug/L total resin acids. Hence, passive transport of resin acids from blood (liver) to bile appears to be usual.

Resin acids are accumulated in tissues of rainbow trout in the same proportions as they occur in the environment (Oikari et al. 1980, 1982). Fox et al. (1977) found that trout exposed to whole kraft mill effluent diluted to 3% (v/v) for 144 hours accumulated 2 ug/g of dehydroabietic acid on a whole-fish basis, while those exposed to a 6% solution for 48 hours accumulated 6 ug/g DHA. These results support the simplistic view that the bioaccumulation factor (BCF) does not differ much between resin acids and that it does not change much over continuous exposure. Oikari et al., 1983 reached the same conclusion.

However, much of the bioaccumulation data of Oikari et al. (1982, 1983) were obtained using exposure times which were relatively short for the size of the fish and the hydrophobicity of the resin acids such that the residue levels in tissues and hence the BCF's may still have been increasing over time and were not constant steady-state levels, which are observed when uptake rate and clearance rates are equal (McLeay & Assoc. 1986). Interpretation of tissue concentrations and BCF data from Oikari et al. 1982, 1983, 1984b, and 1985b is also complicated by the fact that exposure concentrations were decreasing over time and/or were not frequently measured.

Oikari et al. (1984b) found that exposure time was important for rainbow trout exposed to simulated kraft mill effluent for 3, 11, and 30 days. A steady-state equilibrium level in the bile was not observed between the three times of exposure. They hypothesized that three days were not sufficient to reach an equilibrium and that the body clearance rate (and hence time to steady-state) may increase as a result of long-term, low level exposure.

Oikari and Niittyla (1985) demonstrated that bile concentrations of resin acids in trout chronically exposed to dilute kraft mill effluent were linearly related to effluent concentration over the range 0-5% (v/v). No such relationship existed for blood plasma because of rapid clearance to bile during chronic exposure.

Oikari et al. (1985b) found significant quantities of resin acids in blood of trout caged 0.8 km from a kraft mill (1.14-1.38 ug/L total acids), but only trace quantities 3.5 km distant and none 6 km away. However, levels of conjugated resin acids in bile were still detectable 6 km from the mill (Oikari et al. 1980). Resin acid accumulation in the bile has been proposed as a sensitive "bioindicator" of chronic low-level pulp mill pollution (Oikari and Niittyla 1985).

On a whole-fish basis, accumulation of dehydroabietic acid in salmonids reached 20-30 times the surrounding water during acute sub-lethal exposure (Table 3). Much greater bioconcentration factors (BCF) have been reported for major organs such as kidneys, liver, and especially the brain. High concentrations in bile and liver are consistent with a hepato-bilary route of elimination (Oikari et al. 1984a, Kruzynski 1979). No hypotheses as to consequences of DHA accumulation in brain and kidney have been proffered, but the very high BCF's for these organs, suggest that some chronic damage is likely (Fox et al. 1977; Oikari et al. 1980). Modification of, for instance, breeding or feeding behaviour by accumulations of DHA in brain tissue is one obvious possibility (Oikari et al. 1982), but there are no reports of this in the literature. The limited data available suggest a

greater tendency for DHA to accumulate in sockeye salmon compared with rainbow trout (Table 3).

TABLE 3: BIOCONCENTRATION OF DEHYDROABIETIC ACID IN RAINBOW TROUT (except as noted)

Tissue	Concentration In Water (ug/mL)	In Tissue (ug/g)	BCF[a]	Reference
whole body	0.1	2	20	Fox et al. 1977
	0.2	6	30	
	0.4	8	20	
	0.7	9	13	
plasma	1.20	237	198	Oikari et al. 1982
liver		101	84	
kidney		83	69	
brain		37	31	
muscle		16	13	
whole body[b]	0.65	22	34	Kruzynski 1979
bile		18	28	
brain		154	238	
kidney		183	281	
liver		291	447	
skin	0.02	3-4	150-200	Oikari et al. 1983
intestine				
all other organs		1-2	50-100	
whole body	0.65	19	30	Kruzynski 1979
bile		647	996	
brain		620	954	
kidney		278	428	
liver		263	404	

a.Bioaccumulation Factor = concentration in tissue/concentration in water after exposure for: 48h (Fox et al. 1977); 96h (Oikari et al. 1982); 120h (Kruzynski 1979); 30days (Oikari et al. 1983).

b. One fish

c. Sockeye salmon

Rainbow trout exposed to a mixture of resin acids for two days had BCF's very similar to those for dehydroabietic acid alone, which underlines the chemical similarity of this group of compounds. In both cases, the greatest accumulation outside of blood was in the liver, followed by kidneys, brain and muscle (Oikari et al. 1982). In addition to DHA, bioaccumulation in the liver of abietic, neoabietic, pimaric, isoprimaric, levopimaric, palustric and sandaracopimaric acids were reported.

The only example of differential bioaccumulation of individual resin acids is given by Oikari et al. 1984b. After 30 days exposure to simulated kraft mill effluent (approximately 50 ug/L resin acids), rainbow trout accumulated high concentrations of abietic and pimaric acids in the brain (means 22.6 and 18.2 ug/g, respectively), while concentrations of isopimaric and sandaracopimaric were substantially less (1.4-4.0 ug/g) and DHA was apparently excluded.

There are few data available on non-salmonid species. Perch netted from an area exposed to continuous low concentrations of resin acids (1-2 ug/L, except for DHA, 7 ug/L) had blood plasma levels ranging from 42 ug/g (abietic acid) through 22-23 ug/g (isopimaric, pimaric, dehydroabietic) to 7 ug/g (palustric). Bile concentrations were much lower, 2-7 ug/g (except DHA, 17 ug/g) (Oikari et al. 1980). The B/P ratios, all suggest that hepatobilary elimination was occurring by passive diffusion, as typically observed (Oikari et al. 1982, 1984b, Oikari and Niittyla 1985).

Kaiser (1977) reported DHA in a longnose sucker, *Catostomus catostomus* caught about 3 km from a kraft pulp mill discharging to Nipigon Bay, Lake Superior. Fish further from the plant were uncontaminated. Kruzynski (1979) found that DHA was bioconcentrated (whole-body BCF, 20) in an estuarine amphipod, *Anisogammarus contervicolus* after a five day exposure to a high (0.4 mg/L) concentration of DHA. He suggested that bioaccumulation into salmon was possible via ingestion of contaminated food organisms.

3.4 GENETIC EFFECTS

Mutagenesis and carcinogenesis are closely related; that is, most carcinogens are also mutagens (Douglas et al. 1980), although the reverse is not necessarily true. Hence, the two will be discussed together. Nestmann and co-workers have undertaken extensive testing of compounds in pulp mill effluents for mutagenic activity (Nestmann et al. 1979, Douglas et al. 1980, Nestmann and Lee 1983, 1985, Kamra et al. 1983). Most of these tests involve detection of specific mutations in cultures of histidine-dependent strains of *Salmonella typhimurium* (the Ames test, Ames et al. 1975.)

Using this organism, Douglas et al. (1980) tested mutagenicity of eight resin acids (abietic, neoabietic, dehydroabietic, 7-oxodehydroabietic, pimaric, isopimaric levopimaric and sandaracopimaric) and found only one, neoabietic acid was mutagenic, and at relatively low levels. Neoabietic acid produced a dose-dependent response: number of mutants (cells which regained the ability to synthesize

histidine) was constant at doses of 250 ug/plate or less, increased sharply from 250-600 ug/plate, and leveled off asymptotically at higher concentrations (Douglas et al. 1980).

Since neoabietic acid reversed histidine-dependence in *Salmonella* strains which had both base substitution mutations and frameshift mutations (in which successive bases on one DNA strand are shifted by one from the correct base on the opposite strand), it must be capable of causing both kinds of mutations (Nestmann et al. 1979).

An interesting facet of this chemical's mutagenicity is its response to rat liver homogenate (abbreviated S9). Liver microsomes contain enzymes capable of converting pro-mutagens into mutagens that are detectable by the *Salmonella* assay. S9 is added to the test plates to broaden the applicability of the test by permitting mutagen-producing biotransformations that potentially take place within the mammalian body. But *Salmonella* mutations from neoabietic acid are reduced, not increased, by S9 (Nestmann et al. 1979). This implies that mutagenicity of neoabietic acid is altered by liver enzymes, but the exact reactions involved are not known.

The mutagenicity of neoabietic acid was confirmed in later work using enzyme-deficient strains of the yeast, *Saccharomyces cerevisiae* which often detect mutagens that are missed with the *Salmonella* test (Nestmann and Lee 1983). Mutagens at the locus affected increased 2.7 times compared to control cultures. A strong response was also reported for 7-oxodehydroabietic acid, a possible breakdown product of dehydroabietic acid (Servizi et al. 1986) which increased the mutation rate 4.7 times compared to controls. Dehydroabietic acid itself was not mutagenic nor were isopimaric, levopimaric, pimaric or sandaracopimaric acids (Nestmann and Lee 1983). Mutagenicity of palustric acid has not been determined. Both neoabietic and 7-oxodehydroabietic acids produced mutations at the same locus (out of four examined), indicating that they probably work in a similar manner. As in the *Salmonella* assay, mutagenicity of neoabietic acid to yeast was dose-dependent, and increased steadily across the entire range of concentrations used (50-1000 ppb).

Kinae et al. (1981a) tested dehydroabietic acid from samples of coastal sediments off Japan and found no mutagenic activity, relative to background levels, in *Salmonella*. In later work, Kinae et al. (1981b) tested mutagenicity of compounds extracted from liver of sea trout (*Nibea mitsukurii*) exhibiting tumors. This bottom-dwelling fish lives in the coastal areas polluted by pulp and paper mill wastes. Although a number of mutagenic compounds were detected, all of them originally present in polluted sediments, none were resin acids. Table 4 summarizes the known mutagenic properties of resin acids.

Nestmann et al. (1979) have remarked that in their experiments, among the common resin acids, only neoabietic and 7-oxodehydroabietic acids were mutagenic, despite the structural similarity of these compounds. Hence, it must be subtle structural differences, such as the position of double bonds, which are responsible for the presence or absence of mutagenicity. Based on this reasoning, abietic acid has been nominated by the National Cancer Institute's Chemical Selection Work-

TABLE 4: SUMMARY OF MUTAGENICITY OF RESIN ACIDS

Compound	Mutagenicity Bacteria[a]	Yeast[b]	Source
Dehydroabietic acid	No		c
		No	d
		No	e
Pimaric acid	No		c
		No	d
Abietic acid	No		c
Levopimaric acid	No		c
		No	d
Neoabietic acid	Yes		c
		Yes	d
Palustric acid	?	?	Not tested
Isopimaric acid		No	d
Sandaracopimaric acid	No		c
		No	d

a-Ames test with *Salmonella typhimurium*
b-Yeast assay with *Saccharomyces cerevisiae*
c-Nestmann et al. 1980
d-Nestmann and Lee 1983
e-Kinae et al. 1981

ing Group as a candidate for detailed carinogensis bioassay (Sigman et al. 1984). Researchers felt that the close structural similarity of abietic acid with genotoxic neoabietic acid, along with the ubiquity of the former in wood processing wastes and wood products, justified closer scrutiny of abietic acid. As yet no bioassay results have been published.

Five resin acids (abietic, dehydroabietic, isopimaric, pimaric and sandaracopimaric) have been listed as "possible carcinogens" meaning that they are of similar chemical structure to known or suspected carcinogens (Walden et al. 1978).

It must be emphasized that screening tests with microorganisms only indicate potential mutagenesis. They do not prove mutagenesis or carcinogenesis in higher organisms. Defense mechanism of eucaryotic cells and the detoxificiation and im-

mune systems of whole animals may be sufficient to block or repair genetic damage so that the geotoxic potential of, say, neoabietic acid is never realized. Until specific bioassays are carried out on multicellular organisms, the environmental significance of potentially genotoxic resin acids cannot be established.

3.5 FLAVOUR IMPAIRMENT

Pulp mill wastes may cause off-flavours in fish taken from receiving waters (Cook et al. 1972, Persson 1984, Kovacs 1986 and references therein). Many commercial species may develop off odours or flavours when exposed to pulp mill effluent at concentrations of 5% or less (Kovacs 1986). Tainting is usually attributed to a number of compounds, none of them resin acids. Phenolic compounds and their chlorinated derivatives are suspected of being the major contaminating group. Aromatic thiols, formed in the reaction between sulphur and lignin, have also been identified as possible tainters of fish flesh (Kovacs 1986).

Findlay and Naish (1979) did implicate resin acids (along with phenols and volatile hydrocarbons) as possible tainters of rainbow trout exposed to pulp mill effluent near Cornwall, Ontario. However, their approach was strictly correlative: trout were exposed for 48 hours to various process streams from the pulp mill, and the specific chemical composition of each effluent was examined to determine which compounds were consistently present in effluents which caused an off-flavour in fish flesh. Even the very sensitive gas chromatography-mass spectrophotometery analysis they used was insufficient to determine concentrations of contaminants in fish flesh. Hence, it is probable that phenols, many of which are known to taint fish (Persson 1984) were responsible in this instance also, rather than resin acids.

Persson (1984) conducted a comprehensive review of the literature on fish tainting, including that from pulp mill wastes. He lists 57 compounds that have been reported to impair the flavour of fish (Persson 1984: Table 2), of which none are resin acids. The conclusion is that, based on present data, resin acids do not impair the taste or odour of fish flesh.

4. EFFECTS OF RESIN ACIDS ON INVERTEBRATES AND PLANTS

4.1 EFFECTS ON INVERTEBRATES

Resin acid toxicity to non-fish species has not been extensively studied. Most of the extant data are for *Daphnia magna* (Table 5) but even these few data are of limited utility because of incomplete control of environmental conditions, especially pH, and differing duration of tests.

Maenpaa et al. (1968) report 4-h EC50 values for *Daphnia pulex* exposed to a mixture of resin acid sodium salts as 2 mg/L at pH 8.2. The resin acid mixture came from a sulphite mill using spruce, and was dominated by dehydroabietic and abietic acids.

Zanella (1983) tested toxicity of dehydroabietic acid to *D. magna*, and found the pH-dependance of toxicity as for fish (Figure 3, Section 3.1). Forty-eight hour EC50 values ranged from 2.5 mg/L at pH 6.5 to 7.5 mg/L at pH 10 (Table 5). *D. magna* appears to be slightly less sensitive to resin acids than fathead minnows (Figure 3). As well, the *Daphnia* EC50 at pH 7.0 is 3 to 5 times greater than the salmonid LC50 at that pH (Tables 2, 5). However, it is difficult to tell how much of that difference is attributable to comparing 48-hour with 96-hour tests. If they are equivalent, then daphnids are much less sensitive than salmonids to DHA. On the other hand, in the pH range 6.5-7.0, toxicity of DHA to *D. magna* changes rapidly, at a rate of roughly 0.8 mg/L per 0.1 pH unit (Table 5), so small variations in solution acidity will cause large differences in toxicity.

Servizi et al. (1986) report toxicity tests of dehydroabietic and isopimaric acids to *D. pulex* (Table 5). Unfortunately their results are not strictly comparable to those of Zanella (1983) because the former report 96-hour EC50 values, and allowed pH to vary over almost a full pH unit. Their data do indicate that isopimaric acid is considerably more toxic to *D. magna* than dehydroabietic acid, the same as for salmonids.

Servizi et al. (1986) did bioassay DHA and isopimaric acid with sockeye salmon and *D. pulex* under the same test conditions. For both acids, the 96-hour EC50 for salmon was about half that for *Daphnia* (DHA: 2.1 vs. 5.5 mg/L; isopimaric: 0.7 vs. 1.3 mg/L).

It is concluded that daphnids are less sensitive than fish to pure single resin acids. However, earlier studies with DHA and whole mill effluent found that *D. magna* was more sensitive than rainbow trout to these complex mixtures, while the copepod *Cyclops sp.* was relatively tolerant (Eco-Research 1975). These workers also found that the amphipod *Gammarus fasciatus* was killed by untreated ef-

TABLE 5: ACUTE TOXICITY (48-HOUR EC50 IN MG/L) OF RESIN ACIDS TO *DAPHNIA MAGNA*, EXCEPT AS NOTED

Compound	48h-EC50	Test Conditions	Source
Dehydroabietic acid	2.47	pH 6.5, static test temp. = 21°C	Zanella 1983
	6.35	pH 7.0; as above	
	10.8	pH 7.5; as above	
	21.7	pH 8.0; as above	
	38.3	pH 9.0; as above	
	76.9	pH 10.0; as above	
Dehydroabietic acid	5.5[a]	pH 7.2-8.0; static test, 96-hour EC50; 21°C, hardness 85 mg/L	Servizi et al. 1986
Isopimaric acid	1.3[a]	as above	

[a] *Daphnia pulex*

fluent lethal to fish, but not by biologically treated effluent which was non-lethal to fish.

In laboratory bioassays under controlled conditions, midge larvae (*Chironomus tentans*) were more sensitive to whole kraft mill effluent than fingerling rainbow trout (Beak Consultants Ltd. 1974). The amphipod *Gammarus pseudolimnaeus* was less sensitive, and mosquito larvae (*Aedes aegyptii*), which breathe air from above the water surface, were least sensitive of all. Under more natural conditions, where the organisms could take advantage of refugia and avoidance behaviour, trout and *G. pseudolimnaeus* were most sensitive, followed by *C. tentans;* the mosquito larvae were again least sensitive (Beak Consultants Ltd. 1974). It is not known if this sensitivity rank order holds for exposure to resin acids alone.

4.2 EFFECTS ON PLANTS

Although some data are available on effects of pulp mill whole effluents on phytoplankton biomass and primary production, especially in marine environments (McLeay & Assoc. 1986, Dobrocky Seatech Ltd. 1977), there is very little information on resin acid toxicity to plankton or higher plants.

Moore and Love (1977) examined the effects of DHA on productivity of *Cryptomonas erosa* in laboratory cultures (pH 7.5). At the highest concentration tested (500 ppb), DHA significantly reduced production, expressed as uptake of C^{14} per hour, to 90% of that achieved by controls. Inhibition at lower concentrations was insignificant (98 to 99% of control production). A marginally greater reduction (87% of controls) was observed in a second experiment. The concentration found to be inhibiting (500 ug/L) is at the low end of the range for acute lethality to salmonids (500 to 1740 ug/L).

For whole effluents, they also found periphyton to be somewhat more sensitive than phytoplankton: C^{14} uptake by the former was reduced compared to controls by 20-30% more than by the latter at concentrations of 1 to 0.1% effluent (Moore and Love 1977, Figure 4). Whether this difference extends to resin acids is not known.

In contrast to these results, very high concentrations of DHA (10,000 ppb) moderately inhibited production (as measured by increase in dry mass of cells) of the freshwater planktonic alga *Scenedesmus sp.* (Eco-Research Ltd. 1975), but lower concentrations had no effect. There are no further data available to resolve this discrepancy in apparent sensitivity, and no other resin acids have been tested. The effect of pH, which is of critical importance (see Section 3.1) has also not been evaluated.

5. SURFACE WATER CRITERIA FOR RESIN ACIDS

5.1 RATIONALE

Ontario's Provincial Water Quality Objectives and Guidelines are numerical criteria primarily intended to protect all forms of aquatic life at all stages in a life cycle during indefinite exposure to water pollution. Where warranted, an Objective is set to protect wildlife and humans consuming contaminated aquatic species. Similarly, if information indicates that organoleptic effects (taste or odour of water or tainting of fish flesh) may be of concern, a criterion is set to ensure these factors do not affect water use.

A criterion is determined by applying a standard safety factor to the lowest water concentration shown to be deleterious to the most sensitive water use. Generally, a 0.5 factor is applied to a chronic toxicity value if it is available. If only acute (LC50) values are available, a 0.1 or 0.01 factor is applied, the smaller factor chosen for persistent substances. Likewise, an application factor of 0.5 is applied to the lowest organoleptic effect concentration. The two limits, one for toxicity and the other for taste or odour, are then compared and the lower is the surface water Objective.

However, resin acids present an atypical situation. First, they are not known to taint fish flesh (Section 3.5) so that aspect is discounted, at least until more data are available. Second, evaluation of acute toxicity data is complicated by the dependence of toxicity on pH of the water. Third, data on aquatic toxicity, bioaccumulation, and sublethal effects are much more abundant for dehydroabietic acid than for the other seven acids considered here.

Assessment of all the data on resin acids leads to the following conclusions:

- A surface water criterion for any resin acid or acids must specify the pH of the water body to which it applies. The potential for changes in local pH by the effluent itself, and the change in toxicity of resin acids in the pH range 6-8 must be taken into account.

- A surface water criterion may take advantage of the fact that, to a first approximation, all of the resin acids (except DHA) are similar with respect to physical-chemical properties, persistence, bioaccumulation, and toxicity.

- Dehydroabietic acid, because it contains an aromatic ring, has the potential to be more persistent in aquatic systems than the other resin acids.

- Possible toxic interactions between components of pulp mill effluents, such as hypoxia from the combined effects of BOD and resin acid toxicity, need to be considered.

These conclusions, in turn, suggest four possible approaches for a surface water criterion:

- Limit each resin acid independently;
- Limit only dehydroabietic acid;
- Limit the total resin acid content;
- Establish a limit based on some combination of the above.

The first possibility, to establish a criterion for each resin acid separately, is ideal, but is probably not practical or efficient. With the exception of dehydroabietic acid, there are insufficient data to establish defensible water quality Guidelines for each resin acid separately. While it might be possible to develop Guidelines in the absence of complete data, these would inevitably rely on assumed similarities between environmental behaviour and toxicity of the other resin acids with DHA.

There are, however, sufficient data to establish a surface water Guideline for dehydroabietic acid. DHA is not the most toxic resin acid (Section 3.1). It is of particular importance because it is produced in large quantities from every kind of pulping process using all species of softwood trees (Section 2.1); and because it persists and accumulates in sediments. A water quality criterion for DHA would incidentally provide protection against other resin acids. The persistence of DHA in the environment, coupled with its usually high initial concentration, help ensure that if DHA concentrations are low, concentrations of other resin acids will probably be lower, and of less concern. Moreover, analytical procedures would be simplified because only one acid need be detected instead of eight, and that one is not usually obscured by other compounds in the effluent. Finally, there would be less concern that other resin acids present at much lower levels than DHA would be exerting a toxic effect because of the uniformity of toxicities among resin acids.

A surface water Guideline for dehydroabietic acid is feasible and appropriate. However, it would not, by itself, afford complete protection to aquatic life. There is still the possibility in which concentrations of dehydroabietic acid are low, yet concentrations of some other common resin acid, or the sum of several, is high enough to cause a toxic effect. A necessary condition of the third option, that of regulating the total resin acid concentration, is that the acids in a mixture exhibit additive toxicity and do not produce a net toxicity different than that expected from summing the toxicities of the component resin acid separately. There is both direct and indirect evidence to support the contention that resin acid toxicity is additive.

A number of workers have suggested or demonstrated that resin acid toxicities are approximately additive (Leach and Thakore 1976, 1977, Rogers et al. 1979, B.C. Research 1977a, Chung et al. 1979, Walden and Howard 1981, Oikari et al. 1984b).

In an explicit test of additivity, Leach and Thakore (1977) compared toxicities of effluents from de-barking (which contains mostly resin acids; McKague et al. 1977), kraft pulping, caustic extraction and mechanical pulping with estimated toxicities from chemical analysis. They concluded that "agreement was often, though not invariably, within the experimental error of the bioassay and chemical analysis procedures". There was no clear evidence for more-than-additive or less-than-additive cumulative toxic effects. Another study of two British Columbia kraft mills found that toxicity of effluents from various process streams was always additive or less than additive. Supra-additivity was never encountered (B.C. Research 1977b). Chung et al. (1979) also compared toxicity of three pulp mill effluents with toxicity estimated by addition of toxicities of the component chemicals. Agreement between 96-hour LC50 values predicted by adding together the components toxicities and values from bioassays using rainbow trout was within 30% for 76% of the samples. Both less-than and greater-than additivity were observed, although departure from additivity was never extreme (Chung et al. 1979).

Indirect evidence of additive resin acid toxicities comes from their structure-property-toxicity relationships. Resin acid molecules are structurally similar and large (molecular weight greater than 300). The partitioning behavior of resin acids between water and non-aqueous phases suggests that hydrophobicity is a common, non-specific property and from what little is known of dissociation constants, these appear to be similar (McLeay et al. 1979a).

Additive toxicity (concentration addition) is common within groups of structurally similar compounds. Similar compounds will usually exert their toxic effect by the same mechanism, and are absorbed, transported and eliminated by the same systems within exposed organisms. Resin acids are all absorbed across the gill in fish, detoxified in the liver and excreted in bile; they are found in tissue of salmonids in the same relative proportions as they occur in the external medium (Section 3.3); and they all have about equal acute toxicity (Section 3.1). This implies that some common molecular structure or property is responsible for adverse biological effects, although that structure is unknown (Oikari et al. 1983).

Assuming that resin acid toxicity is additive and that the toxicities (ie. LC50 values) do not differ significantly, it follows that the resin acids composing a mixture may be considered interchangeable and it is the total resin acid content that is important. Based on this reasoning, development of a water quality Guideline for total resin acids content seems appropriate.

Protection of aquatic life from resin acids is, therefore, best served by two surface water criteria: one limiting total resin acids content, the other limiting concentrations of dehydroabietic acid. Specific criteria are derived in the next section.

5.2 CRITERIA DEVELOPMENT

5.2.1 Selection of Primary Data

Standards by which to judge the acceptability of toxicity information as primary data, upon which a criterion may be based, are given in "Guidelines for Toxicity Data used in Criteria Development". Those guidelines do not specify that static bioassays are not acceptable; indeed static bioassays are the rule for practical reasons in routine monitoring of effluents by Ministry of Environment personnel. However, here the concern is that the water quality criteria are derived from the most reliable data possible. As described in Section 3.1 and Kruzynski (1977), static bioassays do introduce a systematic factor of 2 error (LC50 overestimated) compared to flow-through assays, and this error would be perpetuated in the final criteria selected. Therefore, it seems prudent to be safe and restrict primary data to those generated by flow-through or semi-static (solution replacement) bioassays. The abundant secondary data by this restrictive definition (Table 2) are still considered in criteria development.

Moreover, because of the pH-dependency of resin acid toxicity, bioassays for which pH was not reported, or was not tightly controlled (range of 0.2 pH units or less) are not considered primary data. Of the studies reporting acute lethality of resin acids listed in Table 2 (Section 3.1), the majority are static bioassays and thus are secondary. Results of Kruzynski (1979), McLeay (1976), and Leach and Thakore (1977), except that for palustric acid, are acceptable as primary data.

There are very few controlled experimental data on chronic toxicity of resin acids. The long-term (30 day) experiment described by Oikari et al. (1983) with dehydroabietic acid and rainbow trout provides the only primary chronic data available.

5.2.2 Provincial Water Quality Guideline for Dehydroabietic Acid

This derivation begins by assuming a fixed pH. Once a suitable Guideline has been developed, the derivation is broadened to include the influence of pH changes. A reference pH of 7.0 was used; this value is typical of neither acidic nor basic conditions, is near the pKa of dehyroabietic acid (7.25; McLeay et al.1979a), and is at or near the pH used to derive primary acute toxicity data. The surface water Guideline should be designed to protect the most sensitive freshwater life. The limited data available on resin acid toxicity to invertebrates and plants suggest that these are no more sensitive than fish and probably somewhat less (e.g. Zanella 1983). Hence, fish are used as the most sensitive aquatic biota.

Leach and Thakore (1977) reported a 96-hour LC50 of 0.75 mg/L for dehydroabietic acid to coho salmon, and Kruzynski (1979) obtained a very similar value for sockeye salmon (0.79 mg/L). The data in Table 2 (Section 3.1) indicate salmon are slightly less sensitive than rainbow trout to resin acid toxicity, but there are no primary data for rainbow trout. There are no reliable data for non-salmonid species, but these are unlikely to be more sensitive than salmonids.

Hence, the coho salmon has the lowest reliable LC50 for dehydroabietic acid and that value (0.75 mg/L) will be used to derive the surface water Guideline.

An application factor for DHA must take into account that the acid has potential to persist and bioaccumulate. Estimated persistence in the sediment column of Lake Superior was 21 years (Brownlee et al. 1977). Both physical-chemical properties and tissue concentration data (Section 3.3) suggest that DHA is bioaccumulative. To allow for these two factors an application factor of 0.01 was applied, to render a tentative Guideline of 0.0075 mg/L, rounded to 0.008 mg/L (8 ug/L). The final step is to check this tentative value for possible sublethal effects. Oikari et al. (1983) present primary data which suggest 20 ug/L is close to the minimum effective concentration for dehydroabietic acid. The tentative Guideline is less than half of that value. The highest bioaccumulation factor reported for DHA is just under 1000 for brain of sockeye salmon (Kruzynski 1979). An ambient concentration of 8 ug/L would lead to a maximum possible bioaccumulation of 8 ug/g of tissue. Since tissue levels would be less in fish exposed to lower concentrations (Section 3.3), that low level is a "worst case". Hence, there are no compelling reasons from chronic toxicity data to revise the tentative Guideline, and 8 ug/L at pH 7.0 is recommended.

Modification of the Guideline to reflect pH changes is based on the assumption that dissociation of the acid, as determined by its pKa, is the factor controlling pH-dependence of toxicity (McLeay et al. 1979a, Zanella 1983). The expected toxicity of DHA may then be inferred from the dissociation curve for the acid, as shown in Figure 4. The curve is sigmoidal, but essentially linear over most of its range. For dehydroabietic acid, McLeay et al. (1979a) found very close agreement between LT50 of dehyroabietic acid to rainbow trout and calculated proportion of un-ionized acid, when plotted on a double log scale. For the range of pH 5-9, beyond which DHA is entirely in the ionized or un-ionized form, the adjusted surface water Guidelines are shown in Table 6. These numbers are based on a strict log-log proportionality of the base criterion 8 ug/L at pH 7.0 with the percentage dissociated acid. They will need to be refined by comparison with laboratory data, especially at the extremes of the range. The Guideline values given decline sharply below pH 7, but above that value they may be relaxed somewhat.

5.2.3 Provincial Water Quality Guideline for Total Resin Acids

The derivation of a Guideline for total resin acids parallels that for dehydroabietic acid. The only difficulty here is that natural resin acid mixtures vary widely in proportions of components, and there is no way to anticipate which compound will dominate at any time or place. The range of toxicities to salmonids is small, roughly 1.5 mg/L in the 96-hour LC50, but it is not insignificant. Therefore, the Guideline for mixtures is based on a value midway between the extremes of isopimaric (96-hour LC50 to coho salmon, 0.22 mg/L) and dehydroabietic (LC50 0.75 mg/L). A value of 0.5 mg/L, about equal to the LC50 of palustric acid, appears to be representative.

TABLE 6: SURFACE WATER GUIDELINES FOR RESIN ACIDS

Receiving Water pH	Concentration (ug/L) DHA	Total Resin Acids
5.0[a]	1	1
5.5[a]	2	3
6.0[a]	2	4
6.5	4	9
7.0	8	25
7.5	12	45
8.0	13	52
8.5	14	60
9.0	14	62

a - lower than established PWQO for pH

Resin acids are all bioaccumulated, but except for dehydroabietic acid, they are not very persistent. Therefore, an application factor greater than 0.01 used for DHA is appropriate. The International Joint Commission recommends 0.05 for non-persistent contaminants. Applying that factor leads to a tentative criterion of 0.025 mg/L or 25 ug/L. This value is slightly greater than the minimum effective concentration for dehydroabietic acid reported by Oikari et al. (1983). If the tenuous assumption that minimum effective concentrations of other resin acids are the same as for DHA is accepted, then the tentative Guideline value of 25 ug/L would not be sufficient to protect aquatic life. However, the persistence of other resin acids is markedly less than that of DHA, so the present, higher value seems proportionally correct.

There is a further difficulty in incorporating pH-dependency of total resin acid toxicity, because dissociation constants for all resin acids are not known (Oikari et al. 1983). However, the dissociation curves for dehydroabietic and abietic acids are almost identical (McLeay et al. 1979a), and the chemistry of these compounds is otherwise similar. Further, the pH-toxicity relationships of DHA and bleached kraft mill effluent are very similar, which provides strong evidence that all the resin acids contained therein have similar dissociation constants. Based on this reasoning, pH relationships for the total resin acids Guidelines are also based on dehydroabietic acid (pKa 7.25), as shown in Table 6. As before, the very high Guidelines at pH 8 or above are suspect, as is the extremely low value at pH 5.0. These values clearly need to be revised with experimental data. The Guideline for total resin acids includes the five most common other than DHA (abietic, neoabietic, pimaric, isopimaric, and sandaracopimaric acids).

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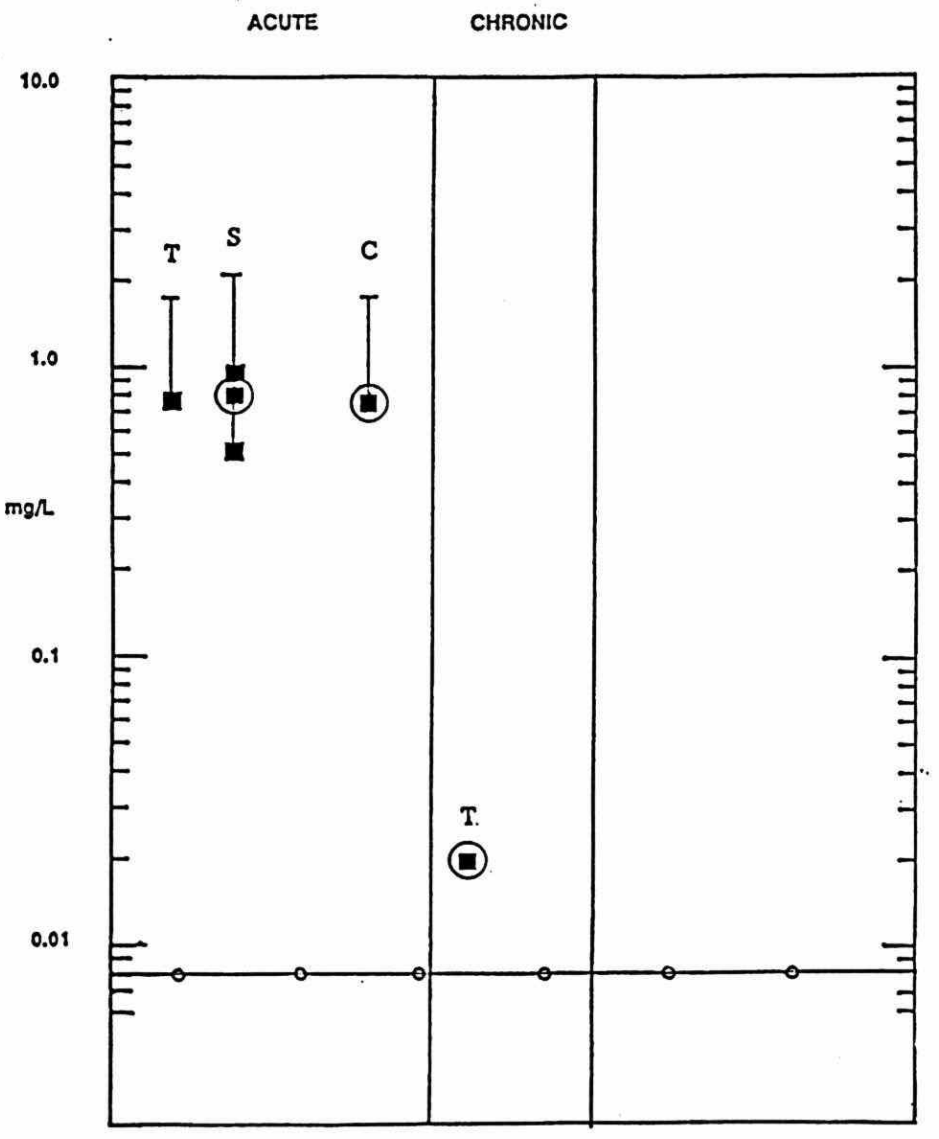


APPENDIX I

TOXICITY AND CRITERION SUMMARY FOR DEHYDROABIETIC ACID

CODES

T RAINBOW TROUT

S SOCKEYE SALMON

C COHO SALMON

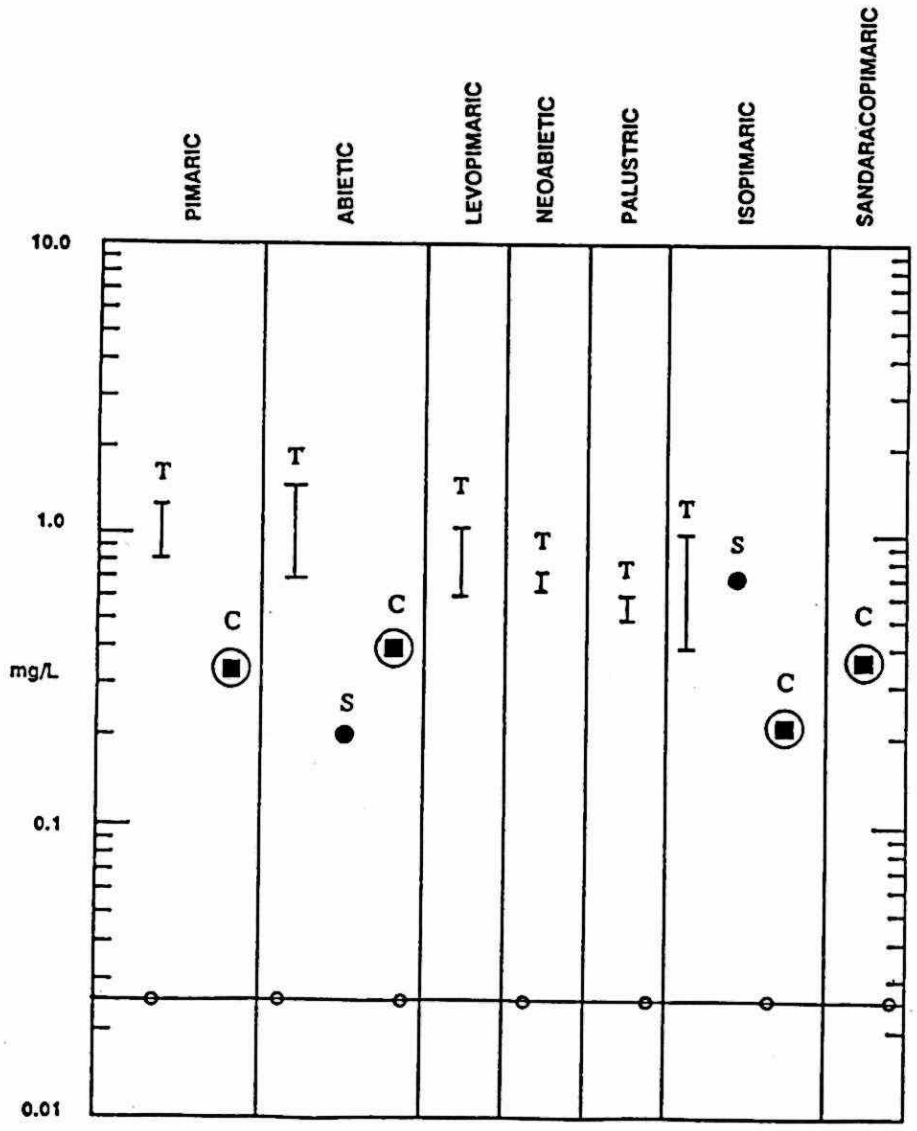
■ PRIMARY DATA

○ CRITICAL VALUE IN CRITERION SELECTION

—●— RECOMMENDED CRITERION

● SINGLE DATUM

I VERTICAL LINES INDICATE DATA RANGES



APPENDIX I I

ACUTE TOXICITY AND CRITERION SUMMARY FOR OTHER RESIN ACIDS

CODES

T RAINBOW TROUT

S SOCKEYE SALMON

C COHO SALMON

■ PRIMARY DATA

⬭ CRITICAL VALUE IN CRITERION SELECTION

—o— RECOMMENDED CRITERION

● SINGLE DATUM

I VERTICAL LINES INDICATE DATA RANGES

APPENDIX 3

SCIENTIFIC REVIEW MEMORANDUM

16 March 1987

TO: Mr. Lewis Yeager, Environmental Applications Group Ltd., 6126 Younge St., 2nd Floor, Willowdale, Ont., M2M 3W7.

FROM: D. George Dixon, Associate Professor, Department of Biology, University of Waterloo, Waterloo, Ontario, N2L 3G1.

RE: Review of the Scientific Criteria Document for Provincial Water Quality Objectives Development: Resin Acids.

This document is a well-written and concise summary of the current state of our understanding of the toxicity of resin acids to aquatic organisms.

The first step in reviewing the document involved establishing the completeness and validity of the data base. Through continuous liason with EAG personnel during preparation of the document, I satisfied myself as to the comprehensive nature of their literature search. The guidelines for the selection of the data used in criteria development were evaluated for scientific defensibility. The guidelines were found to be a valid method of differentiating between reliable and unsound data, hence eliminating work completed using questionable methodology. In order to assess the validity of the data base 12 references (Davis and Hoos, 1975; Iwama et al., 1976; Kaiser, 1977; Kruzynski, 1979; Leach and Thakore, 1977; McLeay, 1976; Moore and Love, 1977; Oikari et al., 1983; Oikari et al., 1985; Rogers et al., 1975; Servizi et al., 1986; Zanella, 1983) were selected at random. These papers were obtained and checked to verify that

1) the data selection guidelines outlined above were followed and 2) that the data were correctly reported in the document. In all of the cases checked, both of the above criteria were met, and the data base is therefore judged to be complete and valid.

After establishing the validity of the data base, I turned my attention to the actual criteria. The tentative criterion for dehydroabietic acid (8 μg l^{-1} with the inclusion of pH as a modifying factor) has been established using standard methods and is both sound and scientifically defensible. The criterion for total resin acids (25 μg l^{-1} with the inclusion of pH as a modifying factor) is also defesible although I agree that it should be provisional based on the absence of chronic data and dissociation constants for the majority of the resin acids.

D. George Dixon, Ph.D.